Endocrine Agents in Breast Cancer:
Studies of Tamoxifen and a New Aromatase Inhibitor.

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Declaration

I confirm that this thesis has been composed by myself.

I acknowledge that the work reported was done by myself, with the exception of immunohistochemistry staining, performed by Miss L MacFarlane, Pathology Department, Western General Hospital, Edinburgh and HPLC runs carried out by Dr J MacCallum, Breast Unit Research Group, Western General Hospital, Edinburgh. I am indebted to several people for advice and assistance in acquiring the techniques necessary to complete the project.

Contents

ABSTRACT

Tamoxifen, a non steroidal partial oestrogen agonist, is the most widely used adjuvant treatment for breast cancer and has been used by millions of women worldwide since its introduction in 1971. Although tamoxifen reduces cancer recurrence and reduces the rate of contralateral cancer, since the late 1980's there has been concern about the effect of tamoxifen on the endometrium. There is evidence of a clear association between tamoxifen use and the development of endometrial abnormalities, with endometrial cancer being increased 2-3 times in women on tamoxifen. In view of this and the recent evidence that prophylactic tamoxifen may benefit high risk women, endometrial screening for women on tamoxifen has been advocated. To date, little work has been done to assess the risk of abnormalities in asymptomatic women to establish whether a screening programme is required and, if it is, what screening tool should be employed.

An initial pilot study was performed to assess the incidence of abnormalities in 120 asymptomatic women with breast cancer on tamoxifen and to establish the most appropriate screening tool from the three assessed, transvaginal ultrasound scanning (TV USS), out-patient hysteroscopy and pipelle biopsy. The pilot study demonstrated that all abnormalities seen at hysteroscopy were identified on TV USS. Following this a further 237 women on tamoxifen and 130 controls had transvaginal ultrasound scanning performed and only went on to out-patient hysteroscopy if the scan was abnormal. There was a statistically significant positive correlation between length of time on tamoxifen and endometrial thickness on ultrasound scan ($p<0.0001$) and women on tamoxifen had a significantly thicker endometrium than controls ($p<0.0001$). 145 women (41%) had an endometrium >5mm on USS and 134 of them had successful out-patient hysteroscopy. 61 women had atrophic endometrium at hysteroscopy giving a 46% false positive scan rate. The remaining women had benign features to explain the ultrasound findings, including cysts in 23 women, oedematous endometrium (23), polyps (21) and submucous fibroids (6). It is concluded that ultrasound is a poor screening tool which gives a high false positive rate. The low frequency of serious endometrial pathology in tamoxifen users and the absence of an ideal screening tool indicates that endometrial screening in asymptomatic women is not worthwhile.

All women in the endometrial study had plasma tamoxifen and its metabolites measured to investigate the relationship between endometrial thickness and tamoxifen levels. There was no correlation between endometrial thickness and blood levels of tamoxifen or any of its metabolites.

Pathology specimens from hysterectomised women on tamoxifen were retrieved to investigate the cystic/oedematous findings seen at hysteroscopy and to assess the effect of tamoxifen on cell turnover. The significant histological findings in tamoxifen users were cystically dilated endometrial glands, low stromal cellularity and the presence or prominence of collagen within the stroma. Immunohistochemistry of hysterectomy specimens for ER and MIB 1 failed to show a significant difference in the expression of either between women on tamoxifen and controls.

Because of the concern regarding tamoxifen's potentially serious side effects, newer more specific antioestrogens and oral aromatase inhibitors are under investigation. Letrozole, an oral aromatase inhibitor, was investigated in 24 postmenopausal women with primary breast cancer and achieved a 92% (22/24) partial clinical response, which is at least as good as results obtained with tamoxifen. This suggests that newer more specific endocrine agents may achieve the effects of tamoxifen without producing any serious endometrial abnormalities.

INTRODUCTION

Tamoxifen is the endocrine therapy of choice for women with breast cancer.

History

Ethamoxytriphetol was the first antioestrogen to be synthesised in 1958 for use in gynaecology and for the treatment of oestrogen dependent breast cancer. The hypothesis was that an antioestrogen could be effective as a 'morning after pill' because it was found to prevent pregnancy in laboratory animals after they had mated. However, trials with ethamoxytriphetol found it to have a low potency and to produce many toxic side effects and so this agent was not further evaluated [Lerner 1990, Wolf 1992].

The main successor compound to ethamoxytriphetol was clomiphene which is an analogue of the oestrogen triphenylethylene and is composed of a mixture of cis and trans isomers. It was found to be more potent than ethamoxytriphetol and clinical trials were initiated. Rather than acting as an antifertility agent, clomiphene induced ovulation and it is currently in widespread use as an ovulation induction agent in the treatment of infertility.

Tamoxifen is structurally very similar to clomiphene and is the trans isomer of triphenylethylene. It is a non steroidal antioestrogenic compound which was first synthesised in Great Britain in 1966. Although the option as a fertility regulator was considered, it was marketed during the 1970's for the induction of ovulation because of its structural similarity to clomiphene [Klopper 1971, Jaiyesimi 1995].

As well as inducing ovulation it was discovered to have antioestrogenic activity in rats and primates. In particular, it was found to suppress carcinogen induced rat mammary tumours so it was postulated it may be effective as an antitumour agent in women with breast cancer.

ICI 46,474 (tamoxifen) was first used in trials for disseminated breast cancer in 1971 and licenced for the treatment of metastatic breast cancer in postmenopausal women in 1973 in Great Britain and in 1977 in USA. Similar licences have been obtained in 110 other countries. Since then tamoxifen has been approved as adjuvant therapy for pre and

postmenopausal women with oestrogen (ER) receptor-positive, node-negative breast cancer, for postmenopausal women with node-positive breast cancer and as adjuvant therapy (in combination with chemotherapy) for premenopausal women with ER positive advanced breast cancer. Tamoxifen is also used to treat breast cancer in men.

Tamoxifen has been used by millions of women worldwide and is currently the most widely prescribed antineoplastic agent for the treatment of breast cancer in the USA and Great Britain.

Pharmacology

Tamoxifen blocks the growth promoting effect of oestrogen in breast tissue by acting as a competitive inhibitor of oestrogen in its binding to cytoplasmic ER (oestrogen receptor). The tamoxifen/ER complex prevents oestrogen/ER mediated gene transcription, DNA synthesis and cancer cell growth. In vitro, tamoxifen inhibits the proliferation of human breast cancer cells by preventing the transition of cells from early G1 phase to mid G1 phase of the cell cycle, resulting in cytostatic effects - cells accumulate in the early stages in the cell cycle while the number of cells later in the cycle diminish. Whether tamoxifen is also cytocidal to breast cancer cells is still debatable [Jaiyesimi 1995]. The mechanism of action in ER negative tumours may vary from that of ER positive breast cancers. Data have shown a decreased proportion of G0 to G1 cells (early stages in the cell cycle) and an increased percentage of cells later in the cell cycle [Reddell 1985, Osborne 1998].

At low concentrations, in tissue culture, the cytostatic effect of tamoxifen can be completely blocked by oestradiol but at higher concentrations of tamoxifen this effect is not reversible by oestrogens. Tamoxifen may inhibit cell replication by methods other than competitive blockade of ER since approximately 10% of ER negative breast cancers respond to tamoxifen therapy [Jordan 1988]. It produces an increase in antibody formation and also inhibits angiogenesis [Gagliardi 1993]. Transforming growth factor-β (TGF-β) has been suggested as a cellular target and is involved in the inhibition of epithelial cell proliferation and maintenance of normal pattern of gene expression in a wide variety of tissues. Loss of response to TGF-β by cells is associated with subsequent malignant transformation. Tamoxifen up-regulates the production of TGF-β by breast cancer cells in vitro and in vivo and may exert part of its antitumour effect by elevation of this cytokine [Jeng 1993, Butta 1992, Colletta 1994]. In addition, tamoxifen mediated reduction in positive growth regulators such as insulin-like growth factors I and II may contribute to the therapeutic effect of the drug. Tamoxifen treatment in vitro and vivo has been shown to reduce serum IGF-I levels [Colletta 1994, Pollak 1992, 1990]. All of these functions of tamoxifen may play a role in the inhibition of tumour growth in ER negative tumours.

Tamoxifen undergoes extensive hepatic metabolism and conjugation. The principle metabolite is desmethyltamoxifen (DMT) and its binding affinity for ER is comparable to

that of tamoxifen. Although present in large quantities, DMT is a relatively weak antioestrogen. Another important metabolite is 4-hydroxytamoxifen (4-OHT) which is present at lower concentrations in plasma than tamoxifen but binds to ER with 25 to 50 times the affinity of tamoxifen such that most of the tamoxifen bound to ER is 4-OHT. 4-OHT is the most potent of tamoxifen's metabolites [Jaiyesimi 1995, Etienne 1989, Wolf 1992]. More recently, Metabolite E has been found to be present in very small quantities and is thought to be the most oestrogenic of tamoxifen's metabolites as it lacks the necessary side chain for antioestrogenic properties [Osborne 1994]. Another metabolite, bisphenol, also lacks the dimethylaminoxy side chain necessary for antioestrogenic properties.

Bliary excretion is the main route of tamoxifen elimination in humans and only a very small amount is excreted unchanged. The half life of tamoxifen and its metabolites ranges from 7-14 days and metabolites can remain in the system for up to 6 weeks following cessation of treatment [Jaiyesimi 1995].

Tamoxifen is a very effective treatment for breast cancer. It increases overall survival and disease free survival in breast cancer patients by decreasing recurrence, reducing the incidence of contralateral breast cancer by 40-50% and by decreasing the incidence of metastases. Initial studies showed that treatment for 2 years was more beneficial than no adjuvant treatment. A subsequent Scottish trial showed a significant reduction in relapses and deaths in those women who received 5 years of tamoxifen when compared with controls [Scottish 1987]. Although there is still debate as to the optimum length of time women should be on tamoxifen, most studies favour prolonged use - greater than 2 years. The Cancer Research Campaign Breast Cancer Trials Group and the Swedish Breast Cancer Co-operative Group have recently both reported that 5 years tamoxifen use is better than 2 years [Swedish 1996, CRC 1996]. Bisset et al concluded there was no evidence to support continuation of tamoxifen beyond 5 years and this is supported by the National Cancer Institute of America [Bisset 1994, Bulbrook 1996]. The optimum duration of tamoxifen treatment remains under ongoing trial investigation, but currently 5 years of tamoxifen use is considered by most to be appropriate [Cameron 1998, Rea 1998, Jordan 1991].

In Edinburgh, at least 5 years is favoured and, until recently, tamoxifen was continued in most women until the time of first recurrence. In a number of cases this has led to very prolonged use of tamoxifen - over 10 years.

Oestrogenic Properties

As well as acting as an antioestrogen on the breast it is evident that tamoxifen has oestrogenic actions on other organs which are beneficial. Studies on laboratory animals have demonstrated that tamoxifen has an oestrogen-like effect on bone and may maintain, or even increase, bone mineral density [Love 1992, Jordan 1987, 1993]. This is potentially beneficial to postmenopausal women as it reduces fracture rate, in particular hip fractures which are a major cause of morbidity and mortality in elderly women [Osborne 1998, Jordan 1993, Wolf 1992].

In addition, paradoxically, tamoxifen causes lipid changes similar to those seen in women taking oestrogen. It reduces low-density lipoproteins and total cholesterol and increases high-density lipoproteins. This could have a significant effect in reducing coronary artery disease in postmenopausal women, a major cause of morbidity and mortality. The Scottish adjuvant tamoxifen trial showed a reduction in the incidence of fatal myocardial infarction in postmenopausal women when compared with control patients [Scottish 1987, Wolf 1992, Jordan 1993, Osborne 1998]. However, this reduction was not seen in the overview analyses of tamoxifen's efficacy in breast cancer [EBCTCG 1998].

Tamoxifen also exerts partial oestrogen-like effects on the hypothalamic-pituitary axis. In postmenopausal women this usually results in a decrease in the levels of circulating gonodotrophins (luteinizing hormone (LH) and follicle stimulating hormone (FSH)), although they remain within the normal postmenopausal range. This is largely irrelevant with regard to the postmenopausal ovary. Serum levels of oestrogen are also reduced in postmenopausal women [Kenemans 1996, Lonning 1995].

Conversely, in premenopausal women who continue to menstruate regularly, oestrogen and progesterone are usually elevated (1 to 4 fold) and LH and FSH levels are unchanged or slightly increased from levels before tamoxifen treatment [Miodrag 1991, Wolf 1992, Uziely 1993, Sherman 1979, Osborne 1998, Cohen 1999(a)]. The theory for this is that tamoxifen reduces the level of circulating oestrogen available to the hypothalamus thus stimulating the secretion of gonadotrophin releasing hormone which in turn stimulates the release of pituitary LH and FSH. However, the fact that LH and FSH levels remain

relatively unchanged, suggests that tamoxifen also acts directly on the ovary to increase steroidogenesis, equalising its agonistic and antagonistic effects to maintain LH and FSH at steady levels. Thyroid-binding globulin (TBG) and sex hormone binding globulin (SHBG) are elevated in pre and postmenopausal women treated with tamoxifen [Szamel 1986].

Endometrial Effects

Not all the oestrogenic effects of tamoxifen are beneficial. Tamoxifen has oestrogenic effects on the endometrium which may lead to a range of endometrial abnormalities including cancer. This has led to some concern especially in view of the recent ongoing trials of the use of tamoxifen for preventing breast cancer. Killackey [1985] was the first to suggest an association between tamoxifen and endometrial cancer in 1985 reporting 3 cases of endometrial cancer in breast cancer patients on tamoxifen. 1 premenopausal and 2 postmenopausal women who had all received 20mg/day tamoxifen for between 7 and 14 months developed endometrial cancer. Since then numerous other cases have been reported and a recent survey of the world literature found 349 cases of endometrial cancer in breast cancer patients who had received tamoxifen [Assikis 1996]. This however must be considered against the background that over 3 million women worldwide take tamoxifen as part of their treatment for breast cancer.

Tamoxifen has also been linked with carcinogenesis in the liver. However, the concern about hepatocellular carcinoma is based entirely on laboratory studies where very high doses of tamoxifen were used. There is no evidence of an increase in the rate of hepatocellular carcinoma in humans at the standard 20mg/day used [Jordan 1995].

Numerous studies carried out in the late 1980's linked tamoxifen with carcinoma of the mouse uterus. The effect of tamoxifen on the growth of the endometrial tumour, EnCa101, was investigated by Gottardis et al [1988]. They developed a useful model to investigate the growth of hormone dependent human tumours in athymic mice by oestrogen supplementation to study the effects of tamoxifen. Implants of endometrial cancer EnCa101 in athymic mice were stimulated by 17β-oestradiol administration but also by tamoxifen. This demonstrates the agonist action of tamoxifen on the uterus in the mouse [Jordan 1991, Gottardis 1988, Satyaswaroop 1984].

The effect of the tamoxifen metabolite 4-hydroxytamoxifen on the proliferation of endometrial cancer has been investigated using the Ishikawa line. 4-hydroxytamoxifen was found to stimulate cell proliferation in this human endometrial cancer cell line [Anzai 1989, Holinka 1991].

Interestingly, Anzai showed that this effect is significantly greater than that of oestradiol on the same cell lines. Other studies including those using athymic mice have shown that the stimulatory effect of tamoxifen is weaker than that seen with oestradiol.

Paradoxically, tamoxifen has been used to treat endometrial cancer. The theory behind this was that it was believed tamoxifen was a pure antioestrogen and that endometrial cancer is a hormone dependent tumour [Swenerton 1979, Tisman 1976, Bonte 1980]. Small studies have reported a favourable effect from tamoxifen but it is now accepted that tamoxifen is not an appropriate treatment for endometrial cancer.

Endometrial Adenocarcinoma

Endometrial adenocarcinoma is the most common gynaecological malignancy and the 4th most common cancer in women accounting for 4-5% of all malignancies. Gynaecological malignancies in general account for 15% of female malignancies [Hoskins 1992, Dewhurst 1986]. It has an age adjusted incidence of 12 per 100,000 women in the UK [Mahboubi 1982] and the disease is more prevalent in caucasian women with the incidence for whites being twice that for non-whites [Hoskins 1992, Koss 1984]. The peak period of incidence is in the 55 to 65 age group but 25% of cases occur in premenopausal women [Tindall 1987]. The incidence of endometrial cancer in the USA peaked in 1975, probably as a consequence of the use of unopposed oestrogen HRT [Dewhurst 1986, Mahboubi 1982], but there has been a continuous decline since 1979. However, it continues to cause 4000 deaths per year. Risk factors for endometrial cancer include late menopause and early menarche (ie long menstrual history), nulliparity, obesity, diabetes mellitus and polycystic ovaries (all of these imply elevated endogenous oestrogens). Nulliparous women who make up between 24-31% of patients with endometrial cancer have 2-3 times the risk of developing the disease compared with women with children [Dewhurst 1986].

The true cause of endometrial cancer is unknown and it can arise in normal, atrophic or hyperplastic endometrium. Studies suggest that at least two different mechanisms are implicated in its aetiology:

(i) a history of exposure to unopposed oestrogen, endogenous or exogenous. These tumours begin as endometrial hyperplasia and progress to cancer. They tend to be better differentiated and have a better prognosis. 1% of cystic hyperplasia, 14% of adenomatous hyperplasia and 25-40% of atypical hyperplasia develop into cancer [Hoskins 1992, Dewhurst 1986].

(ii) spontaneous tumours arising from a background of atrophic or inert endometrium. In 75% of women the tumour is confined to the uterine corpus and it tends to spread over the endometrium to form a diffuse malignancy rather than invading the myometrium (which is a rather late event). It tends to present early with bleeding, usually postmenopausal bleeding as the majority of women who develop endometrial cancer are postmenopausal (75%) [Hoskins 1992, Dewhurst 1986].

Tumours are staged by the FIGO (International Federation of Gynaecology and Obstetrics) system which was first introduced in 1971 and recently updated (1988):

Stage I	A -	Confined to the endometrium
	B -	Invasion to <½ myometrium
	C -	Invasion to >½ myometrium
Stage II	A -	Involving uterus and endocervical glands only
	B -	Cervical stromal invasion
Stage III	A -	Spread outside the uterus invading serosa and/or adnexa and/or positive peritoneal cytology
	B -	Metastases to pelvic and/or para-aortic lymph nodes
Stage IV	A -	Tumour invasion of bladder and/or bowel mucosa
	B -	Distant metastases including intra-abdominal and/or inguinal lymph nodes

[Hoskins 1992, Dewhurst 1986, Tindall 1987]

Most tumours are early stage (Stage 1), because these early cancers cause bleeding and therefore the majority have a good prognosis (80% overall 5 year survival) [Jordan 1996 Mahboubi 1982].

Stage	Percentage	% 5 Year Survival
1	74	72.3
2	13.5	56.4
3	6.2	31.5
4	2.8	10.5

[Shaw, Souter & Stanton 1997].

Tumours are also graded G1-3 with regard to degree of differentiation. Grade I is well differentiated with 5% or less of a non-squamous or non morular solid growth pattern and Grade 3 is poorly differentiated with more than 50% of a non-squamous or non-morular solid growth pattern.

Although adenocarcinoma is the most common type of endometrial cancer, there are other tumours which are typically more aggressive - namely, serous carcinoma, papillary carcinoma, clear cell carcinoma and sarcomas which include mixed mullerian tumours. Sarcomas are associated with a history of pelvic irradiation.

Tamoxifen and Endometrial Carcinoma

An association between breast cancer and endometrial cancer, regardless of tamoxifen use, has been suggested because of the common risk factors [Adami 1987, Gaglione 1989, Fotiou 1998]. However, in addition to this background risk, and following Killackey's first report, there have been a number of retrospective and prospective studies and non-controlled case reports suggesting an association between tamoxifen use and the development of endometrial cancer.

The first report of a higher frequency of endometrial cancer came from the Stockholm trial [Rutqvist 1987, 1995]. In this trial, 1846 postmenopausal breast cancer patients were randomised to receive 40mg/day tamoxifen or placebo for 2 years. Those who were disease free at 2 years were further randomised to an additional 3 years tamoxifen or placebo. In the tamoxifen group 17 endometrial cancers were found compared with 3 in the control group. The 1995 update revises numbers to 23 in the study group and 4 in the control group. This study suggested that long-term tamoxifen resulted in more endometrial cancers.

The biggest of the tamoxifen trials is the National Surgical Adjuvant Breast and Bowel Project Study (NSABP B-14)[Fisher 1994]. They compared rates of endometrial cancer and other cancers in 2843 patients with node-negative, oestrogen receptor (ER) positive, invasive breast cancer who were randomly assigned to 20mg/day tamoxifen or placebo and in 1220 tamoxifen treated patients registered subsequent to randomisation. In the tamoxifen group 23 endometrial cancers were found compared with 2 in the control group. Most were stage 1 and therefore of good prognosis but 4 women in the tamoxifen group died from their endometrial cancer. This trial reported an absolute risk of tamoxifen induced cancer of 1-2 cases annually per 1000 women treated and a relative risk of 2-3 times greater than that seen in women with breast cancer not on tamoxifen. They also showed a fairly constant rate of endometrial cancer over time on the drug.

Four other big trials have failed to show an increased risk:-

- The Scottish trial involved 661 tamoxifen treated women and 651 controls. In the tamoxifen treated group 4 women developed endometrial cancer compared with 2 in the control group over the same time period (5 years or until time of first relapse). Women received 20mg per day of tamoxifen. This trial concluded that there was no association between tamoxifen use and endometrial cancer as there was no difference in the frequency of endometrial tumours in both groups [Scottish 1987].

- The Christie trial, in which 282 patients were randomised to receive 20mg of tamoxifen daily for 1 year, detected 1 tumour in each group (tamoxifen and control) after a follow-up period of 13 years [Ribeiro 1988].

- The NATO (Nolvadex Adjuvant Trial Organisation) trial also failed to show an increased incidence of endometrial cancer in the tamoxifen group (564 patients) compared to controls (567 patients) over an 8 year follow-up [NATO 1988].

- Katase et al [1998] investigated gynaecological follow-up in 825 breast cancer patients (279 on tamoxifen and 546 controls). They found 4 women on tamoxifen and 9 control women who subsequently developed endometrial carcinoma and concluded there was no increase in incidence with tamoxifen use.

There have been 8 major adjuvant trials, none designed specifically to look at the association between tamoxifen and endometrial cancer, but all have retrospective data from double blind randomised trials allowing them to draw conclusions. The cumulative incidence, from all these trials of endometrial cancers in women treated with tamoxifen, is 0.9% compared with 0.2% in the control groups, which means an extra 7 women per 1000 who took adjuvant tamoxifen developed endometrial cancer. Although not all trials have reported an increased incidence, it is likely that the numbers in some of these trials were not sufficient to detect the small but significant difference in incidence between tamoxifen treated women and controls.

Table 1 summarises these 8 trials.

Table 1

Summary of eight major adjuvant tamoxifen trials showing incidence of endometrial cancer in patients and controls.

Study	Tamoxifen Treated Patients		Controls	
	No. Patients	**No. Developed Endometrial Cancer**	**No. Patients**	**No. Developed Endometrial Cancer**
Copenhagen	164	2	153	0
Christie	282	1	306	1
NATO	564	0	567	0
Scottish	661	4	651	2
ECOG-1178	85	1	83	2
Stockholm	931	17	915	3
Toronto	198	0	202	1
NSABP B-14	1419 1220	15 8	1424	2
Total	5524	48	4301	10
	(0.9%)		(0.2%)	

[Jaieyesimi 1995]

As well as the large randomised trials comparing tamoxifen with placebo there have been numerous other case reports and case control studies of endometrial cancer development in women on tamoxifen.

Table 2 summarises some of these reports.

Table 2

Case reports and case control studies of endometrial cancer in association with tamoxifen use.

Author	No. of Cases	Dosage(mg)	Duration(mths)
Killackey	3	20	7-14
Pons	1	30	10
Hardell	11	40	12-120
Fornander	13	40	24-60
Dauplat	2	20-30	18-24
Mathew	5	20	50-96
Malfento	7	20	18-48
Atlante	4	40-60	24-60
Neven	1	20	36
Rodier	1	40	8
Segna	11	10-20	5-84
Uziely	2	n.r.	>24
Osborne	4	n.r.	12
O'Neill	1	20	60
Mignotte	20	20	29-102
Deprest	1	40	96
Le Boudec	4	20-30	3-30
Spinelli	3	20	24-36
Palacois	1	20	104
Teshima	4	20-40	24-96
Samelis	1	20-40	24-156
Dew	1	20	48
Seoud	6	20	0 >48
Lahti	1	20-40	6-95
Ismail	2	20-40	36-85

Author	No. of Cases	Dosage(mg)	Duration(mths)
Peters-Engl	8	20-40	6-60
Jose	1	20	72
Fotiou	5	10-30	>24

[Sismondi 1994, Peters-Engl 1996, Jose 1995, Fotiou 1998, O'Neill 1992, Mathew 1990]

What is not clear from the data is whether any increase in the number of cases of endometrial cancer represents a true increase in endometrial cancer in tamoxifen treated patients or results from lead time bias, ie an increase in detection of lesions that would otherwise have remained silent [Barakat 1995, Dew 1995]. In none of these trials did women undergo initial endometrial screening to give a baseline before starting tamoxifen nor were they randomised with respect to major risk factors for the development of endometrial cancer. In support of this Horwitz et al [1981] reported occult endometrial cancer at autopsy in a general population five times higher than the reported rate for the same geographical area over the same time period [Wolf 1992]. In addition, current studies fail to give clear information about the time period over which tamoxifen causes problems. Some studies have looked at relatively short periods of tamoxifen use, ie 0-2 years and whether true cause and effect can be drawn from studies of such short duration is debatable. Although the Stockholm trial followed women for 5 years and concluded that endometrial cancer was more frequent in patients who continued tamoxifen beyond 2 years, in their study the majority of cancers were actually detected in patients who had been treated with tamoxifen for 2 years or less.

The Christie trial, looking at short term tamoxifen, failed to show an increase in endometrial cancer with tamoxifen use. Very few studies to date have looked at long term tamoxifen, 5-10 years or even 10+ years use. The Scottish trial did follow women on long term therapy and failed to show an increased incidence of endometrial cancer, although with longer follow-up there is a suggestion that those randomised to 5 years or longer are at greater risk of endometrial cancer. Studies also vary in the dose of tamoxifen given - 20mg/day is the current recommended dose in the UK and the US but different centres within Europe have used varying doses from 20mg up to 60mg daily.

What is also not clear is whether tamoxifen acts as a true carcinogen or as a promoter of existing lesions. Concern is such that in the USA tamoxifen has been labelled as a carcinogen by the FDA [Jordan 1993].

From the literature, it is accepted that tamoxifen is associated with some increased risk of endometrial cancer (of an order of 2-3 times) [Fisher 1994, Friedman 1994] but the benefits of tamoxifen in the adjuvant treatment of breast cancer and in reducing contralateral cancers far outweigh the risks of reported endometrial abnormalities.

Although most studies have reported that tumours which develop following tamoxifen use are well differentiated at early stage and have a good prognosis [Seoud 1993, Barakat 1995, Cuenca 1996, Katase 1998], some reports suggest that tamoxifen is linked to more aggressive endometrial cancer. In the reported literature from 1984-1995, there were 18 mixed mullerian tumours and 9 sarcomas [Assikis 1996, Altaras 1993, Seoud 1993, Hardell 1988 (b)] as well as high grade, poorly differentiated adenocarcinomas which have developed in women who have taken tamoxifen.

This information is summarised in Table 3.

Table 3

Total number of endometrial cancers and high grade tumours in the reported literature, 1984-1995.

Endometrial carcinomas	349
Mixed Mullerian tumours	18
Sarcomas	9
Patients	
Postmenopausal	200
Premenopausal	2
Duration of tamoxifen therapy	
<2 years	91
>2 years	108

NB Information was not available for all patients regarding menopausal status or duration of tamoxifen therapy.
[Assikis 1996]

Since then there have been other sporadic reports of high grade tumours [Clement 1996, Dallenbach-hellweg 1995].

Sarcomas which include carcinosarcoma (mixed mullerian and mixed mesodermal tumours), and leiomyosarcoma are rare in the general population and account for <5% of all uterine cancers. They have an annual worldwide incidence of 0.5 - 3.3 cases per 100,000 women and, unlike endometrial carcinoma, occur more commonly in black women. The peak incidence age is 70 years and previous pelvic irradiation appears to be a risk factor. It is uncertain whether they arise from endometrium or myometrium but they are highly aggressive with early lymphatic and haematogenous spread. Consequently survival is poor with a 5 year survival of <10% [Tindall 1987, Hoskins 1992].

Magriples et al [1993] reported a retrospective cancer registry search in which 67% of patients (10 women) in the tamoxifen group had poorly differentiated endometrial carcinomas (including adenosquamous carcinoma) or carcinoma associated with poor

outcome (papillary serous carcinoma, clear-cell carcinoma, mixed mullerian carcinoma). They also reported that patients in the tamoxifen group were much more likely to die from their endometrial cancer. Of interest in this study, all women received 40mg/day tamoxifen. The Scottish trial reported 4 endometrial tumours in the tamoxifen group studied and all were sarcomas [Stewart 1989]. Malfento [1990] described 7 new cases of endometrial cancer in breast cancer patients on tamoxifen, all of whom received 20mg/day tamoxifen for an average of 28 months prior to diagnosis of endometrial cancer. Of the 7 cases, 2 were grade 3 (poorly differentiated) and 1 patient died from her endometrial cancer.

Associated Pathology

Tamoxifen has also been linked with other endometrial abnormalities including polyps, endometrial hyperplasia and endometriosis. Endometrial hyperplasia has been reported in postmenopausal breast cancer patients taking tamoxifen. Gal et al [1991] initially obtained endometrial biopsies from 38 women on tamoxifen and found 7 (18%) with endometrial hyperplasia ranging from simple to complex with atypia. They went on to prospectively monitor women on tamoxifen by means of endometrial biopsy and found new hyperplastic changes in 3/11 women. Other studies support the finding of hyperplastic changes in women taking tamoxifen [Uziely 1993, De Muylder 1991, Lahti 1993, Cohen 1993 (a), Cohen 1994 (c)]. Cross and Ismail [1990] describe a case report of endometrial hyperplasia in an oophorectomised woman on tamoxifen.

Polyps have also been associated with tamoxifen therapy, both in isolation and in association with hyperplastic elements [Seoud 1993, De Muylder 1991, Nuovo 1989, Ismail 1994 (a), Corley 1992 Ugwumadu 1993, Cohen 1992, McGonigle 1996]. Endometrial polyps in the general population are rarely associated with neoplastic change, with only a 0.5% chance of cancer being present within a polyp but Ramondetta et al [1999] reported 5 cases of tamoxifen associated endometrial polyps with foci of carcinoma within them.

Endometriosis (another oestrogen dependent condition) and hyperplasia of endometriotic foci have been reported in several women taking tamoxifen [Ford 1988, Cano 1989, Le Boudec 1991, Morgan 1994]. Endometriosis is a disease characterized by the presence and proliferation of endometrial tissue outside the uterus. This has raised the possibility of induction of malignant change in the endometriotic foci and not just the endometrium of women on tamoxifen [Buckley 1990].

In addition, the growth of uterine fibroids has been documented with tamoxifen use. [Rullo 1993, Ugwumanda 1994, Dew 1995].

As well as acting on the uterus, tamoxifen also has an oestrogen-like effect on vaginal epithelium. This is not surprising because embryologically the uterus and upper 2/3rds of the vagina are derived from the mullerian duct system. It would therefore seem logical that

if tamoxifen has an effect on the uterus, it should also affect the vagina. Boccardo [1981, 1984] and Ferrazi [1977] obtained vaginal smears and determined the karyo-picnotic index (KPI); this is the relation of mature superficial cells to mature intermediate cells and is one of the most common indices for cytohormonal evaluation. Administration of oestrogens to postmenopausal women generally induces cellular maturity which increases KPI values. In postmenopausal women, tamoxifen increases KPI values indicating a mild to moderate oestrogenic effect [Lahti 1994]. Vaginal pH was measured in postmenopausal women on tamoxifen by Miodrag et al [1991] and found to be significantly reduced towards levels typically found in premenopausal women. Ferrazi [1977] found a zero KPI value before tamoxifen treatment and reported smears returned to atrophic within 2 months of tamoxifen withdrawal.

Friedrich et al [1998] investigated the effect of tamoxifen on vaginal and cervical epithelium in postmenopausal women on tamoxifen use and showed an increase in the maturation index with increasing length of tamoxifen use compared to controls. They also showed an apparent increase in endocervical cell hyperplasia and metaplasia with tamoxifen use. Reassuringly, both this study and that by Rayter et al [1994(b)] and Gill et al [1998] failed to show any evidence of cervical dysplasia in tamoxifen treated patients.

It has been suggested that tamoxifen may also affect the ovaries reflecting common risk factors and may affect pre and post menopausal ovaries differently [Ewertz 1990, Cohen 1996(b)]. Functional cysts in premenopausal women, most of which resolved spontaneously, have been reported and have been postulated to be secondary to supraphysiological oestradiol serum levels which are seen in premenopausal women on tamoxifen [Shushan 1996, Cohen 1999(a)]. Data on cyst development in postmenopausal women is scanty and requires further investigation. One small study suggested an increased risk of cysts in postmenopausal women also [Cohen 1996(b)]. Vaginal ultrasonography is a useful tool for assessing the endometrium and ovaries simultaneously and could be used in further studies to address this issue [Lindahl 1997, Carter 1991].

There are therefore a number of problems associated with tamoxifen use but clearly not all women who take the drug develop problems. Kedar et al [1994] showed in their breast cancer prevention trial that most women (61%) who took tamoxifen had an atrophic endometrium at follow-up. The difficulty is predicting those women who will experience an oestrogen-like effect from tamoxifen use, although the cumulative dose may be important [van Leeuwen 1994, De Muylder 1991]. To date no method has been devised for predicting which women will be affected.

Endometrial Screening

The effect of tamoxifen on the endometrium has led to the question of whether there should be screening for women on tamoxifen to detect endometrial abnormalities. If screening is to be implemented, the best method both of picking up abnormalities early and patient tolerability is yet to be established. Also under debate is how often women should be screened - once only, or on a regular basis (ie yearly) and should women be assessed prior to commencing tamoxifen. These all have enormous cost implications when taking into consideration the number of women on tamoxifen.

Tamoxifen is being investigated as a preventative agent for women at high risk of developing breast cancer and trials are currently under way. Recent preliminary results from one trial have shown a reduction in breast cancer incidence with prophylactic use of tamoxifen and it now has a product licence in the USA for prevention of breast cancer in high risk women. The implications for screening this group of women also need to be considered as annual transvaginal ultrasound examination and biopsy of any ultrasound detected lesions has been recommended by some [Bissett 1994, Ross 1995, Barakat 1995].

According to the WHO, a screening programme should fill the following criteria:

- the disease should be reasonably common and serious and its natural history should be known.
- it should have a latent period during which time it would be appropriate to screen.
- treatment should be available and treatment at the time of diagnosis should improve prognosis.
- the test should be valid and acceptable to patients.
- the cost of screening should be economically balanced in relation to expenditure on medical care as a whole.

[Wilson 1968].

There are a number of potential endometrial screening methods available:

- Ultrasound scanning - abdominal and transvaginal
- Hysteroscopy - out and in-patient
- Pipelle endometrial biopsy
- Dilation and curettage (D & C)

Ultrasound scanning is probably the easiest and least invasive of the screening techniques and the transvaginal route is the scanning method of choice because the image obtained is far superior to that of the transabdominal technique [Fleischer 1990, Mendelson 1988]. This is principally because of the short probe to target distance, allowing the use of higher frequency transducers. It is accepted that an endometrial thickness in a postmenopausal woman measuring 5mm or less is normal [Fleischer 1990, 1997, Granberg 1991, Bourne 1995, Goldstein 1990] whilst in premenopausal women the thickness of endometrium depends on when in the menstrual cycle the scan is performed, but anything up to 10mm in the late secretory phase is normal [Dodson 1995, Dijkhuizen 1996]. An abnormally thick endometrium may suggest a precancerous or cancerous lesion, endometrial hyperplasia or a polyp, none of which have specific morphologic features to allow distinction between them [Varner 1991, Lewit 1990] but a thick endometrium with hyperdense echoes and no distinct demarcation of endometrium/myometrium, fluid within the cavity, an enlarged cavity and uterus are all suspicious of endometrial carcinoma in a normal postmenopausal population [Carter 1991, Blumenfield 1996]. Bourne's [1995] review of 1700 symptomatic postmenopausal women reported no endometrial cancer within a uterus whose endometrium measured <5mm. Other studies support a very high correlation between normal endometrial measurement on ultrasound scan and atrophic endometrium at histology [Nasri 1989, 1991, Schoenfeld 1990, Granberg 1991, Possati 1994, Karlsson 1994, Mencaglia 1995].

A number of studies have been carried out assessing the use of transvaginal scanning mainly in symptomatic women on tamoxifen and comparing this to other screening methods. However, several workers have shown that thick cystic endometrium and alterations in the uterine vessel vascularity - a decrease in the resistance index (RI) of vessels similar to that seen in endometrial cancer - are frequent findings in women taking tamoxifen [Kurjak 1990,

Bourne 1991, Achiron 1995(a), (c)]. They suggest that, in patients on tamoxifen, transvaginal ultrasound findings may be misleading because thickening of the endometrium is not always associated with epithelial disease [Anteby 1992, Hulka 1993, Goldstein 1994, Bese 1996]. It has also been suggested that tamoxifen may exert an echogenic and sonolucent effect on the endometrial stroma and myometrium without necessarily causing epithelial disease [Cohen 1993, Seoud 1993]. Achiron [1995(a)] found this cystic appearance in 44% of women studied and 2 large studies of postmenopausal women on tamoxifen therapy failed to show any correlation between an endometrium measuring >5mm in thickness and abnormal pathologic findings [Uziely 1993, Cohen 1993]. It may be that cut-off values for endometrial thickness used in the general population do not apply to women on tamoxifen and apparent increases in endometrial thickness on ultrasound scan may be misinterpreted as abnormal.

In terms of a screening tool, ultrasound scanning is simple and cheap to perform, with a low incidence of side effects but, from the literature, may have problems in terms of interpretation of results, sensitivity and specificity.

Hysteroscopy and pipelle biopsy have also been suggested but are more invasive than sonography and so may not be ideal screening tools.

Pipelle biopsy is a blind procedure and is potentially less sensitive than hysteroscopy. Pipelle fails to obtain an adequate sample of endometrium in 20-30% of women (higher in postmenopausal women) [Guisa-Chiferi 1996, Gupta 1996, Weber 1997, Batool 1994].
D & C is also a blind procedure though it permits more thorough sampling of the endometrium. Even with D & C, false negative rates of 10-15% have been cited and one group found that in 60% of cases less than half the uterine cavity was curetted [Stock 1975, Word 1958, Grimes 1982, Brooks 1988]. Several workers have therefore suggested hysteroscopy is the 'gold standard' investigation for women [Finikiotis 1990, Gimpelson 1995, Dijkhuizen 1996, Towbin 1996, Gupta 1996]. Hysteroscopy ensures that the whole uterine cavity is visualised and so sampling can be directed. Finikiotis [1989] suggested that in a series of 523 symptomatic women, based on hysteroscopic observation, D & C was unnecessary in 56% and that where abnormalities were present, more were observed visually on hysteroscopy than were detected by D & C. Specificity and positive predictive

value of hysteroscopy has been quoted at near 100% [Parasnis 1992, Mencaglia 1995]. Although this is the best method available, it is not an ideal screening tool because of its invasive nature.

There have been several studies comparing transvaginal ultrasound scanning to out-patient hysteroscopy, mainly in non breast cancer non tamoxifen women, to compare their ability to pick up abnormalities [Towbin 1996, Maia 1996, Widrich 1996, Gupta 1996, Emanuel 1995, Karlsson 1994, Indman 1995] but few have assessed patient tolerability for each procedure and also whether patient tolerability affects the choice of screening method employed.

Finikiotis [1993] reported that 70% of patients undergoing hysteroscopy report side effects but that preoperative explanation of the type, severity and frequency of side effects reduces patient anxiety at the time when side effects occur. Towbin et al [1996] reported that 141/149 patients who underwent out-patient hysteroscopy were "comfortable" during the procedure. Downes et al [1993] went further and did score patient acceptability in 100 premenopausal women attending for out-patient hysteroscopy. They used a score of 0-10, 0= no pain/completely acceptable and 10= worst pain imaginable/completely unacceptable and found that the mean maximum pain experienced during the procedure was 3.25 (S.D. 2.08) and the degree of acceptability was 3.02 (S.D. 1.96). Very few patients (3%) said they would prefer a general anaesthetic if the procedure was to be performed again. De Jong et al [1990] also aimed to assess patient acceptability of the out-patient procedure but did so under paracervical block which could be argued to be unnecessary and more painful than the procedure itself. In most of the women they studied, the level of discomfort was described as tolerable but in 2 out of 152 women the procedure was abandoned because of severe discomfort.

With the advent of narrow flexible hysteroscopes the need for paracervical block should diminish and perhaps the only indication would be where cervical dilatation is required in a patient with cervical stenosis [Nagele 1996]. This most commonly occurs where the patient is nulligravid, nulliparous and postmenopausal. The main source of discomfort arises from insufflation with the CO_2 gas and not from passing the instrument through the cervix.

There is still no concensus about whether asymptomatic women on tamoxifen should be screened nor what the ideal screening tool would be.

Thus, although it is well accepted that there is an association between tamoxifen use and endometrial abnormalities, including cancer, the majority of women have few problems on tamoxifen. Little work has been done to assess who is at risk and whether they can be identified prior to problems arising. This would give a target group to be screened because at present workers are divided as to the merits of screening all women on tamoxifen trying to pick up the few it will affect. The arguments against this centre on the cost implications of regular screening and the large numbers of invasive procedures screening generates.

Plasma Levels

Little work has been done trying to identify which women taking tamoxifen are at risk of endometrial abnormalities. None of the studies to date have looked at whether plasma levels of tamoxifen are important and relate to endometrial effects. The same daily dose of tamoxifen is given to all women regardless of height and weight and therefore body mass index (BMI) and few have examined whether this significantly affects plasma tamoxifen levels. Other oncological drugs are given in different doses related to BMI so it would seem logical that this should also be done with tamoxifen. Indeed, whilst 20mg/day of tamoxifen is now the recommended dose, as noted above, in some studies 40mg or even 60mg/day has been used. Some researchers believe that cumulative dose may be important [Fornander 1989]. In the UK the recommended dose has decreased from 30mg/day to 20mg in the last decade after the efficacy of 20mg was demonstrated in advanced disease. However, this dose was derived empirically.

We know that with oral administration steady state plasma levels are reached in 3 to 5 weeks. Tamoxifen is extensively metabolised in the human by hydroxylation and N-demethylation and in plasma the main metabolite is desmethyltamoxifen (DMT). The ratio of DMT to unchanged tamoxifen at steady state is normally greater than 1.0 [Fried 1994]. Tamoxifen is excreted in urine and bile and has a half life of approx 10 days [de Vos 1992].

There are several methods described in the literature for the determination of tamoxifen and its metabolites but there have been problems with recovery and analysis of the target compounds. The analysis of tamoxifen is difficult because of its low plasma concentration and extreme light sensitivity.

The basic principle of plasma determination of tamoxifen and its metabolites is by extraction and high performance liquid chromatography (HPLC). Organic and solid phase extraction methods have been used with varying success. Until recently, most pre HPLC methods were time consuming leading to problems with photo degradation of tamoxifen, but new quicker methods have been devised to alleviate this. Organic extraction yields efficiencies of 60-90% whereas traditional solid phase extraction only 30%. However, a rapid and simple solid phase extraction method has been designed in Edinburgh which gives a reliable efficiency of approx 60% [MacCallum 1996].

To enhance sensitivity because of the low plasma concentrations of tamoxifen and its metabolites, photochemical activation can be employed, by which tamoxifen, a triphenylethylene, is converted by ultraviolet (UV) radiation to a phenanthrene. This is a unique property of tamoxifen's molecular structure which allows it to react under UV irradiation. Thus levels can be estimated by UV fluorescence after conversion to phenanthrene derivatives, either before or after HPLC [MacCallum 1996, Kikuta 1988, Fried 1994, De Vos 1992].

Tamoxifen Side-Effects

In addition to endometrial problems, women report a number of other symptoms related to tamoxifen use. These include hot flushes/sweats and weight gain (probably the two most commonly reported symptoms), fluid retention, vaginal dryness and vaginal discharge, decreased sexual interest and low energy. Although these symptoms may not be classed as "major", for some women they are very distressing and intolerable. A few find the risk of stopping their tamoxifen before the recommended time more acceptable than having to carry on with daily living coping with these symptoms. However this accounts for less than 5% of women on tamoxifen [Jordan 1996, Wolf 1991].

In most cases there are options to try and alleviate these distressing symptoms including megestrol acetate, clonidine, venlafaxine or prozac. If vaginal symptoms are caused by significant vaginal atrophy, it is acceptable to try a short course of Vagifem, an oestrogenic pessary with minimal systemic absorption.

These symptoms can be particularly distressing in premenopausal women with the relatively sudden cessation of oestrogen especially if combined with oophorectomy.

The NSABP-14 trial compared tamoxifen versus placebo and investigated how commonly these symptoms occurred in both pre and postmenopausal women and whether they were confined to tamoxifen use. They found that hot flushes, vaginal discharge and irregular menses were experienced more commonly in the tamoxifen group (Table 4). However, many reports are anecdotal and it may be that symptoms such as weight gain and decreased sexual interest are a reflection of the diagnosis and surgical treatment of breast cancer and the psychological problems associated with that rather than subsequent tamoxifen treatment.

Hepatocellular cancer and ocular toxicity are 2 more serious side effects which have been reported with tamoxifen use. Hepatocellular cancer has only been reported in laboratory studies using very high doses of tamoxifen and is of theoretical risk only in vivo. Occular toxicity has been reported in vivo but is rare and usually reversible following cessation of tamoxifen [Kaiser-Kupfer 1978, Jordan 1995, Nayfield 1996].

Table 4

NSABP-14 5 Year Tamoxifen Trial (20mg/day) vs Placebo. Reported Symptoms.

Adverse Effect	Tamoxifen (n=1424)	Placebo (n=1420)
Hot Flushes	63.9%	47.6%
Weight Gain (>5%)	38.1%	40.1%
Fluid Retention	32.4%	29.7%
Vaginal Discharge	29.6%	15.2%
Nausea	25.7%	23.9%
Irregular Menses	24.6%	18.8%
Weight Loss (>5%)	22.6%	18.0%
Skin Changes	18.7%	15.3%

New Antioestrogens

Breast carcinomas in women can be separated into two types - those which are hormone dependent and which contain oestrogen receptors and those which are hormone independent and do not express the oestrogen receptor. The majority of tumours, particularly in postmenopausal women are hormone dependent and treatments which lower circulating levels of oestradiol or block its action in the breast result in tumour regression.

Tamoxifen has been the primary agent of choice for women with postmenopausal breast cancer with ER positive large operable or metastatic breast cancer for the last 20 years and has been shown in patients with tumours which are rich in oestrogen receptor to produce partial or complete responses ranging from 79-85% [Howell 1998]. It also has other effects on the breast decreasing recurrence, decreasing contralateral cancer development and decreasing the rate of development of metastases but, as described, there are unwanted effects particularly on the endometrium, and much work has been done to find effective alternatives to tamoxifen.

This has come in three forms:

(i) newer SERM's (selective oestrogen receptor modulators), eg raloxifene, toremifene, idoxyphene.

(ii) more specific antioestrogens, eg faslodex.

(iii) aromatase inhibitors.

(i) **SERM'S**

Raloxifene is the most promising of the new SERM's (Selective oEstrogen Receptor Modulators) which were originally titled non steroidal antioestrogens. Raloxifene was developed for preventing osteoporosis and potentially for reducing the risk of cardiovascular disease on the basis of its oestrogen-like activity [Delmas 1997, Walsh 1998]. In addition, unlike tamoxifen, raloxifene may reduce the risk of breast cancer without increasing that of endometrial cancer. In a trial evaluating the effect of raloxifene on fractures in postmenopausal women it was found to produce a 76% reduction in the incidence of breast cancer [Cummings 1999]. As initial results are promising, further trials of raloxifene versus tamoxifen are currently underway assessing breast cancer incidence in pre and postmenopausal women [Jordan 1999(b)]. The potential effect of raloxifene on the endometrium is yet to be established [Jordan 1999(a)], although this is promising because raloxifene has been found to produce complete or near complete blockade of oestrogenic effect in the uterus of castrated rats [Bryant 1995] and in chemoprevention trials halved the endometrial cancer risk compared to a placebo group [Cummings 1998].

(ii) **Specific Antioestrogens**

Faslodex, ICI 182780, is a pure antioestrogen which appears to be more specific and more potent than tamoxifen in vitro and in vivo because of its different mechanism of action. Rather than acting as a competitive inhibitor (like tamoxifen), faslodex substantially reduces the cellular content of the ER by decreasing the half-life of the protein [Anderson 1996]. It appears to have minimal side-effects in particular on the uterus and has been shown in rat models to bind to the ER with high affinity, acting as a complete antagonist [Anderson 1996, Wakeling 1991]. Howell et al [1996] reported no apparent effect of treatment with faslodex on the endometrium or the hypothalamic-pituitary axis of 19 patients with advanced breast cancer resistant to tamoxifen. The pure antioestrogens are therefore promising future alternatives which are under further investigation.

(iii) **Aromatase Inhibitors**

Aromatase is an enzyme which is required for the conversion of androgens to oestrogen as part of the steroid pathway (Fig 1). In premenopausal women, the

primary source of oestradiol is the ovary, where the enzyme aromatase catalyses conversion of androstenedione to oestrone and testosterone to oestradiol. The amount of oestradiol produced depends on luteinising hormone (LH) which controls the amount of substrate present and follicle stimulating hormone (FSH) which regulates the activity of aromatase. Following the menopause, oestrogens are primarily derived from peripheral aromatisation of adrenal precursors of which the largest concentration is androstenedione. Although several factors can influence the output of androstenedione in postmenopausal women, eg stress and obesity, the amount of oestrogen produced is not controlled primarily by substrate availability but by the magnitude of aromatase activity present. Aromatase is found predominantly in fat, liver and muscle, the amount of fat being the major regulator of extraglandular aromatase [Miller 1982, 1989]. Following aromatisation of androstenedione to oestrone, 17β hydroxysteroid dehydrogenase converts oestrone to oestradiol in peripheral tissue. Since most peripheral tissues are not amenable to either surgical or radiological ablation as treatment for breast cancer, oestrogen deprivation needs to be based on therapies which destroy androgen producing tissues or drugs which inhibit the enzymes involved in oestrogen biosynthesis, eg aromatase. Drug induced oestrogen blockade is much more attractive in that it is potentially reversible and produces minimal side effects [Jonat 1990, Miller 1995].

The concentrations of major oestrogens differ markedly between the circulation and the breast. Evidence for this is that:

(i) tissue concentrations of oestradiol are similar in pre and postmenopausal women despite the marked reduction in peripheral oestradiol levels following the menopause.

(ii) in postmenopausal women endogenous levels of oestrogen are significantly higher in the breast compared with the circulation, the tissue : plasma ratio for oestrone is approximately 3 and that for oestradiol is 20, and

(iii) oestrone and its sulphate levels predominate over those of oestradiol in the circulation but levels of oestradiol are similar to or higher than oestrone in breast tissue. The differences in oestrogen within mammary tissue could be because of -

(a) selective uptake of specific oestrogens from the circulation against a concentration gradient, or

(b) active synthesis or metabolism within the breast [Miller 1989].

In vivo studies have demonstrated significant uptake of oestrone and oestradiol into breast tumours. Although receptor binding is a major determinant of tissue oestrogen levels, several studies have supported the hypothesis that breast tumours can synthesise oestradiol locally [Miller 1982]. Androstenedione may be converted to oestrone by the aromatase enzyme and oestrone may be produced from oestrone sulphate via the sulphatase enzyme (Fig 2). A final common pathway is then necessary to convert oestrone to oestradiol through the 17β hydroxysteroid dehydrogenase enzyme [Jonat 1990]. The enzymes for each of these reactions have been demonstrated to be present in human breast cancer tissues and the biological importance of tumour aromatase is that aromatase inhibitors can block oestrogen production irrespective of the site of synthesis, whether it be glandular or peripheral and will directly block oestradiol synthesis within a tumour. Clinically, useful inhibitors should have the potential to suppress oestrogen levels beyond those achievable by destruction of any individual endocrine gland [Miller 1989]. Oestrone sulphate, oestrone and oestradiol all arise from aromatised androstenedione, so inhibition of aromatase is a key target for reducing the amount of oestradiol available to stimulate breast tumour growth.

The first aromatase inhibitor used in clinical practise was aminoglutethamide but it was used originally without the knowledge that it had antiaromatase properties. It was introduced as a form of medical adrenalectomy for the treatment of metastatic breast cancer. It was, however, also found to block peripheral conversion of androgens to oestrogens and to suppress circulating oestrogens. It was found to be a non specific inhibitor and affected other cytochrome P450 systems, blocking cholesterol side chain cleavage and thus corticosteroid production (cortisol and aldosterone). Aminoglutethamide markedly lowered plasma and urinary oestrogen levels (by 50-80%) and clinical trials revealed rates of breast tumour regression similar to conventional antioestrogens. In postmenopausal women with advanced breast cancer it produces 90-95% inhibition of aromatase and response rates similar to those reported for other endocrine therapies [Santen 1999].

However, whilst clinically effective, aminoglutethamide is not an ideal aromatase inhibitor because of its non-specificity, side-effects and the requirement for corticoid replacement when prescribed at higher doses because it interferes with glucocorticoid synthesis. A wide variety of newer and more specific aromatase inhibitors have been developed and third generation aromatase inhibitors include drugs such as anastrozole, letrozole and exemestane which are substantially more potent than aminoglutethamide at inhibiting in-vitro aromatase activity, are more selective and have substantially fewer side effects [Hamilton 1999].

To date the trend has been to use aromatase inhibitors as second or third line hormone therapy, ie in advanced disease because of their low specificity and side effects but the interest in the newer generation of aromatase inhibitors stems from the fact that they appear to be more potent and much more specific. They suppress whole body aromatization by >95% and oestrogen levels by at least 80% and these effects can be achieved without measurable influences on other steroid hormones [Geisler 1995].

Neo-adjuvant (primary medical) treatment has been used to treat large operable and locally advanced breast cancers but this has been mainly in the form of chemotherapy. Few centres have evaluated neo-adjuvant endocrine therapy in hormone sensitive large operable and locally advanced breast cancer. In postmenopausal women the most common neo-adjuvant endocrine agent used has been tamoxifen and in those with oestrogen receptor positive tumours high response rates have been reported and significant reductions in tumour volumes seen over a 3 month period [Keen 1997].

However, with the concern regarding tamoxifen's side effects and the development of the newer potent, specific aromatase inhibitors, studies are in progress to investigate the effectiveness of aromatase inhibitors as neo-adjuvant therapy for patients with locally advanced and large operable breast cancers.

Figure 1

Steroid Pathway.

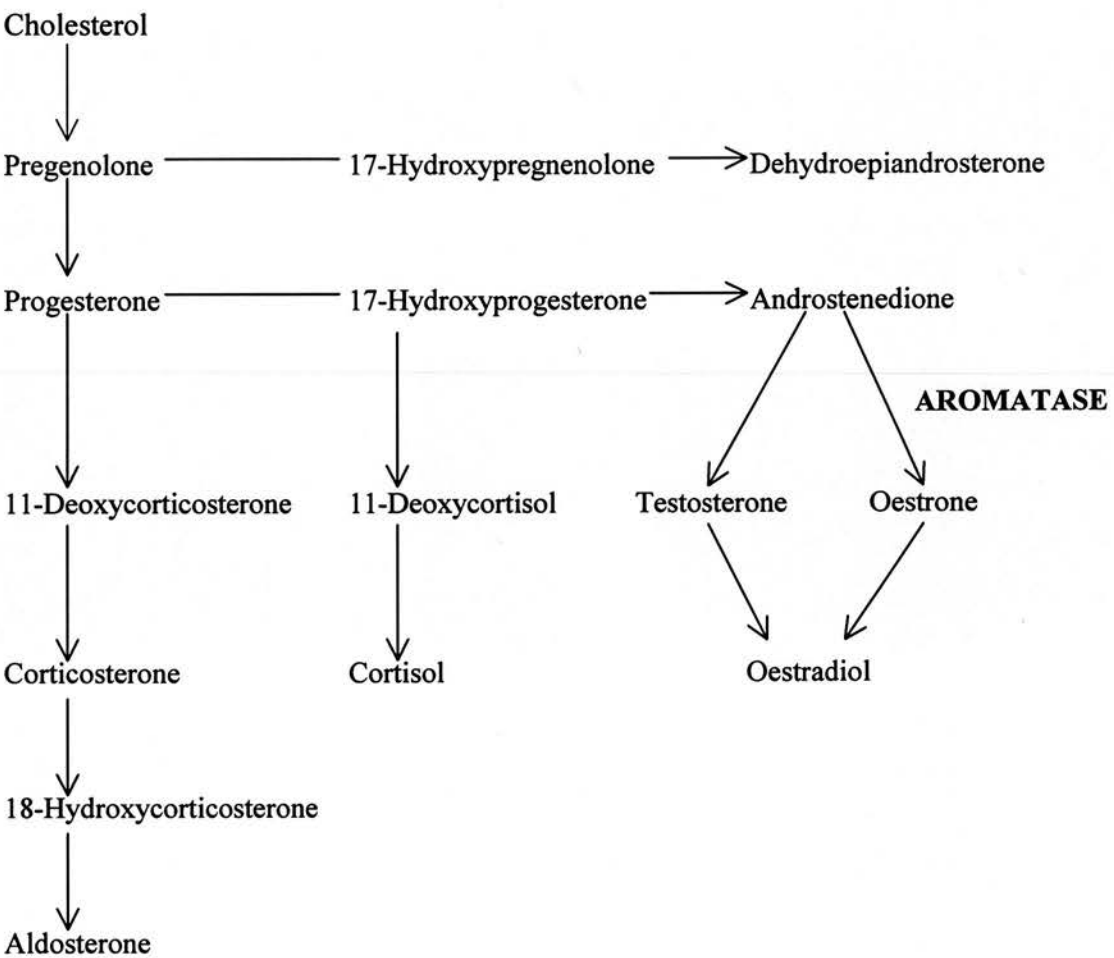
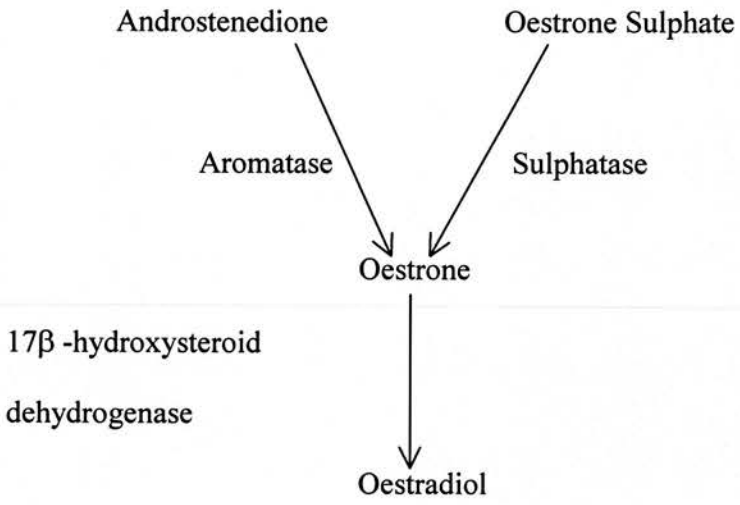


Figure 2

Routes of Oestradiol Production.



Thesis Aims

Tamoxifen has an undisputed role in the treatment of breast cancer but early studies suggested a link between tamoxifen use and the development of endometrial abnormalities, including cancer. Subsequent research has attempted to establish the extent and determine the frequency of endometrial abnormalities. Little work has been done to assess the risk of abnormalities in asymptomatic women on tamoxifen and therefore to establish whether screening for these women is required. If a screening programme is to be considered, the ideal screening tool has yet to be established. There exists an ideal opportunity in Edinburgh to investigate whether screening is of value as women, until recently, remained on tamoxifen until the time of first recurrence of breast cancer, consequently giving a large potential study population of women receiving tamoxifen for variable lengths of time, some for over 15 years.

Of particular interest is the standard dose of tamoxifen taken by all women, 20mg/day, regardless of body mass index. Little work has been undertaken relating plasma levels of tamoxifen to the subsequent development of endometrial abnormalities.

The aims of the present series of studies can therefore be summarised as:

- to determine the incidence of endometrial abnormalities in asymptomatic patients with breast cancer, taking tamoxifen;
- to investigate the relationship between length of time on tamoxifen and endometrial abnormalities;
- to investigate the relationship between blood levels of tamoxifen and endometrial abnormalities;
- to investigate the relationship between body mass index and plasma tamoxifen levels to assess whether all patients require the same dose;

- to evaluate the following screening tools - transvaginal ultrasound and out-patient hysteroscopy in terms of picking up abnormalities and patient tolerability;

- to investigate histology and markers of cell turnover in thickened endometrium;

- to assess the incidence of other side effects on tamoxifen;

- to investigate alternative endocrine agents.

METHODS

Patient Population

All patients were recruited from the long term follow-up clinic in the Breast Unit at the Western General Hospital. Patients attend 6 monthly for the first 2 years following diagnosis and treatment of breast cancer, then yearly thereafter. Patients on tamoxifen were identified from the clinic list prior to their attendance and were approached immediately following their visit. All patients in the study group were disease free and on tamoxifen as adjuvant treatment for breast cancer and had been on tamoxifen at the time of recruitment for between 5 months and 16 years duration. 357 women on tamoxifen, both pre (42) and postmenopausal (315) were recruited over the study period. All women had an intact uterus at the time of recruitment.

Pilot Study

The first 120 women recruited were asked to take part in the pilot study which involved having a transvaginal ultrasound scan, a pipelle biopsy and out-patient hysteroscopy performed. 150 women were asked and 120 agreed to these investigations. Consent was obtained for all procedures.

The aim of the pilot study was to identify the best method of screening in terms of picking up abnormalities and tolerability of the procedure.

An ultrasound scan was performed on all women and pipelle biopsy was performed immediately following the scan.

Patients returned within the next 10 days to have out-patient hysteroscopy performed. It was not possible to perform all three investigations at one visit because pipelle biopsy may cause bleeding, rendering it impossible to view the cavity with the hysteroscope. Hysteroscopy is not ideally done prior to the pipelle because it uses insufflation with CO_2 gas in order to view the cavity. Pipelle works by suction and prior uterine insufflation with CO_2 renders this difficult. The scan was performed in the Mammography Department and the hysteroscopy in the Day Bed Area at the Western General Hospital, logistically making it difficult to have all investigations performed at the same visit. Therefore, it was necessary for patients to attend on two separate occasions.

Study Group

Following the pilot study, the remaining 237 women recruited (from 260 approached) underwent ultrasound scanning and patients with an abnormal scan went on to out-patient hysteroscopy. Out-patient hysteroscopy was again performed at a separate visit and consent was obtained for all procedures performed.

Controls

200 women with a diagnosis of breast cancer never having received tamoxifen were approached and asked to act as controls. They were identified from the database of the Edinburgh Breast Unit and from long term follow-up clinics. Where women were identified from the database, a letter was sent to them explaining the study and asking if they would agree to take part. Where they were identified from the long term clinic, they were approached directly after their clinic appointment. 130 agreed to take part and all women were asymptomatic at the time of investigation. They consented to ultrasound scanning only as it was not anticipated that many of this group would require further investigation for abnormalities.

All GPs were notified of patients entering the trial and were sent a copy of the patient information sheet. GPs were thereafter notified of abnormal results and the subsequent action taken.

Patients requiring investigation beyond hysteroscopy were referred to the Eastern General Hospital under the care of Dr J B Scrimgeour. Further investigation consisted of D & C in 35 women and oophorectomy in 2 women.

Statistical Analysis

Statistical analyses were conducted using the Instat software package (Graphpad Software Inc, San Diego, CA).

Transvaginal Ultrasound Scanning

Experience in transvaginal ultrasound scanning was gained over a 4 month period from Dr B B Muir, Consultant Radiologist. All scans were performed using an ATL Apogee 800 ultrasound machine (Advance Technology Laboratories UK Ltd, Letchworth, UK) with a transvaginal probe specially purchased for this project.

All women recruited into the study had transabdominal pelvic ultrasound performed using a 2-5MHz curved array probe immediately prior to transvaginal ultrasound to screen for major pelvic abnormalities as the transvaginal probe has a narrow field of vision. Patients were asked to attend with a full bladder which was necessary for the transabdominal approach. Following the transabdominal scan, patients were asked to empty their bladder prior to the transvaginal scan which was performed using a 5-8 MHZ endocavity (transvaginal) probe.

All scanning was performed in a specific ultrasound room with the patient lying on a couch. A gynaecologic examining table was not available so patients were asked to sit on a pillow in order to tilt their pelvis forward making the scan easier. This allowed scanning of the anterior pelvis which requires the transducer handle to be passed down toward the floor so that the transducer tip is projected upward, directing the ultrasound beam into the anterior pelvis. The vaginal probe was covered with a condom (ultra strong condom as recommended for protection against infection, in particular HIV infection). A sonic coupler was placed in the condom and KY jelly applied to the outside of the condom. The probe was then passed into the vagina and advanced to the level of the vaginal fornix to begin the scan.

A sonic coupler is needed to decrease the acoustic impedance as sound leaves the transducer and enters the body. Even a small layer of air between the transducer and the body results in marked sound attenuation and artefacts. To acoustically couple the transducer to the vaginal mucosa, jelly was placed in the condom. Additional lubricant is placed on the surface of the condom to serve as an acoustic coupler between the condom and the patient [Dodson 1995].

In each case the uterus was identified initially and its length, width and thickness measured in millimetres by first directing the sound beam anteriorly and posteriorly in the pelvis, giving an anterior posterior pelvic (AP-pelvic) plane image and then rotating the transducer through 90 degrees to direct the sound beam across the pelvis from side to side giving a trans-pelvic (T-pelvic) plane image. All measurements were made using the machine's electronic callipers. The endometrium was then measured, double thickness, in millimetres in both the T-pelvic and AP-pelvic position. The endometrium is visualised within the uterus as a central echo and the whole endometrium was evaluated and measured at its thickest part. Premenopausal endometrium can measure up to 10mm in the secretory phase but the upper limit for postmenopausal endometrium is 5mm. Both ovaries, where present, were visualised and checked for the presence of cysts by moving the transducer lateral to the uterus in the T-pelvic plane. Premenopausal ovaries are generally easy to identify because of their size and the presence of anechoic follicles within them. Postmenopausal ovaries can be more difficult to identify but this study was interested in the detection of ovarian cysts, which, like ovarian follicles, are easily identifiable if present. Any other abnormalities were also noted, eg free fluid in the Pouch of Douglas, intracavity fluid or fibroids [Dodson 1995].

Ultrasound images of the uterus and endometrium in the AP-plane and T-plane were frozen and a hard copy was exposed onto single sided emulsion x-ray film using a multiformat camera. All films were retained for future validation purposes.

The probe was decontaminated as recommended by the manufacturer between patients by washing with Travisept (Chlorhexidine Acetate BP 0.015% and Cetamide Ph.Eur.0.15%) and once weekly the transducer was disinfected by soaking in Travisept for 20 mins.

Of the 120 women in the pilot study, the first 10 in whom thickened endometrium was detected were re-scanned by Dr B B Muir to confirm the investigator's findings. In all 10 cases, thickened endometrium was confirmed. Thereafter, Dr B B Muir re-scanned cases only where the investigator was unsure of the findings and requested confirmation.

Hysteroscopy

Out-patient hysteroscopy was performed in the Day Bed Unit at the Western General Hospital. A trained nurse assisted with the procedure. A 3.6mm diameter Olympus HYF-P flexible hysteroscope (KeyMed (Medical & Industrial Equipment) Ltd, Livingston, Scotland) was used which did not require a local anaesthetic cervical block. Uterine insufflation using an Olympus uteroflator was with CO_2 gas or, on occasion, saline. Training required to perform hysteroscopy was obtained under Dr J B Scrimgeour and Dr J A Milne, both Consultant Gynaecologists working at the Eastern General Hospital, Edinburgh. Initially, hysteroscopy was performed with consent on women attending the Eastern General Hospital as day cases for general anaesthetic hysteroscopy and D & C, usually for investigation of abnormal bleeding/postmenopausal bleeding. Once competent at the technique, further experience was gained by attending out-patient hysteroscopy sessions run by Dr J A Milne at Leith Hospital, Edinburgh. Initial study hysteroscopies were supervised and validated by Dr J B Scrimgeour. Subsequently hysteroscopies were recorded on video and reviewed at the end of each session by Dr J B Scrimgeour. Where abnormalities were detected or where hysteroscopy could not be performed, patients went on to day case D & C and hysteroscopy for further assessment under general anaesthetic at the Eastern General Hospital, Edinburgh.

Out-patient hysteroscopy took approximately 30 minutes to perform and all patients were fully aware of potential side effects. The most common side effect is crampy lower abdominal pain which is similar in nature to 'period pain', whilst a few patients experience shoulder tip pain caused by the CO_2 gas permeating out of the fallopian tubes into the abdominal cavity and tracking up the paracolic gutters, irritating the underside of the diaphragm. The only potentially serious side effect is cervical shock which may result from passing the instrument through the cervix. This leads to bradycardia and hypotension and can result in loss of consciousness. A facial oxygen mask and oxygen supply and intravenous atropine was readily available at all times for the treatment of cervical shock.

All patients underwent hysteroscopy in the lithotomy position. Vaginal examination was performed before each procedure to determine size and position of the uterus. This was a clean rather than sterile procedure although the hysteroscope was sterilised between patients

using Nu-cidex. A vaginal speculum was passed and the cervix and cervical os visualised. A uterine sound was passed to determine cavity length and direction. The cervix was grasped with a vulsellum, if necessary, and the hysteroscope passed through the cervical os into the uterine cavity. Several elderly women required minor cervical dilatation using cervical dilators before the hysteroscope could be passed. In 8 women cervical dilatation was not possible because of cervical stenosis and the procedure was abandoned. All 8 women proceeded to D & C for further investigation.

Insufflation took approximately 30 seconds and once a good view was obtained the uterine cavity was inspected thoroughly from uterine fundus to internal cervical os and cervical canal as the hysteroscope was withdrawn. Both ostia were identified in all cases.

The procedure was visualised using an Olympus camera attached to the hysteroscope and a 21" Sony colour monitor. All hysteroscopies were recorded and retained on video for validation purposes.

Where appropriate, an attempt was made to obtain endometrial specimens using a Sharman curette and fixed in 10% buffered formaldehyde solution prior to being sent to pathology. Biopsy forceps were used in an attempt to obtain material where the investigator was unable to obtain specimens by means of the Sharman curette. Unfortunately, few specimens were obtained by either of these methods and in cases where specimens were essential to exclude serious underlying pathology, women proceeded to D & C.

Once the procedure was finished, patients were asked to remain lying down for 5-10 minutes to recover and allow any discomfort to subside and thereafter they remained in the out-patient department for at least 30 minutes before going home.

All patients were informed of the result of the hysteroscopy before leaving the department.

Pipelle Biopsy

Pipelle biopsy was attempted in the 120 women who took part in the pilot study. This was done following the ultrasound scan and involved passing a standard plastic disposable speculum into the vagina and visualising the cervix. Once visualised the pipelle was passed through the cervix and specimens obtained by applying suction. Specimens were fixed in 10% buffered formaldehyde solution and sent to the Pathology Department, Western General Hospital, for review.

It became apparent early in the study that pipelle biopsy was not successful in a large number of women and that few satisfactory specimens were obtained. The specific problems encountered were:

1. Many of the women were postmenopausal and elderly and therefore suffered from vaginal atrophy. The disposable speculums used are a standard size and, as such, were too big for these women. They found the procedure too uncomfortable to permit pipelle biopsy. The speculum used for the hysteroscopy were Cusco bivalve speculum which are metal and variable in size and were used because there was the facility to sterilise them between cases. Had the investigator had the facility to sterilise the smaller metal speculum in the scanning room, this method may have been more successful. This was not possible because of the conditions attached to the use of Nu-Cidex. The financial constraints of the study did not allow for the purchase of large numbers of Cusco speculum which would require to be sent to, and await return from, CSSD (Central Stores and Supplies Department) within the Western General Hospital for standard steam sterilisation.

2. Visualisation of the cervix and cervical stenosis was a further problem. The women were not in an ideal position (unlike for the hysteroscopy where they were in lithotomy on a gynaecological table with their legs on leg rests) to allow easy visualisation of the cervix and to attempt dilation of the cervix where necessary, so whilst all pipelle biopsies were attempted, only 59% were successful. A successful pipelle was classed as one where the cervix was adequately visualised and the pipelle passed through the cervical os to allow the procedure to be performed whether or not tissue was subsequently obtained.

Tissue Specimens

One of the aims of the study was to investigate markers of proliferation in endometrial specimens from women on tamoxifen. The aim was to obtain these specimens by means of Sharman endometrial biopsy curette (Dupae Health Care, Leeds, UK) taken during hysteroscopy or pipelle biopsy. Unfortunately insufficient samples were obtained by these methods to make this feasible.

Specimens were subsequently identified from ISD Scotland (Information & Statistics Department), Trinity Park House, Edinburgh. A list of all breast unit patients with a diagnosis of breast cancer in the preceding 10 years was obtained from the SMR (Scottish Morbidity Records) 6 database (Cancer registration) and supplied to ISD and was linked to their SMR 1 database (General acute in-patient and day case discharges) to determine those women who had also had a hysterectomy performed after the diagnosis of breast cancer had been made.

From hospital notes it was then possible to determine whether or not they had been taking tamoxifen at the time of their hysterectomy and from most notes the pathology details of the hysterectomy could be determined. For ease of retrieval of specimens, only women who had their hysterectomy in the Edinburgh area (Royal Infirmary, Western General, Eastern General); St John's Hospital/Bangour Hospital, Livingston; Borders General Hospital, Melrose and Roodlands Hospital, Haddington, were included. No patients were approached but all consultant gynaecologists at the above hospitals, where a gynaecology unit is still present, were contacted to obtain permission to retrieve gynaecology notes where required. Identifying women who had received their gynaecological surgery in the Edinburgh area also implied that they were most likely to be followed up from their breast cancer in the regional centre serving these areas - the Edinburgh Breast Unit, Western General Hospital, Edinburgh, making retrieval of oncology case notes possible.

By combining SMR 1 and SMR 6 data, 195 women were identified in the Edinburgh and surrounding area. 134 sets of case notes were retrievable and all were studied by the investigator. 48 women were identified on tamoxifen at the time of their hysterectomy and a further 19 women were identified to act as controls (with a diagnosis of breast cancer but

not receiving tamoxifen at the time of hysterectomy).

The pathology specimens for 48 women (34 study women and 14 controls) were retrieved with the help of Dr Alistair Williams, Consultant Pathologist, Royal Infirmary, Edinburgh and Dr Alistair Lessels, Consultant Pathologist, Western General Hospital, Edinburgh. Haematoxylin and Eosin (H & E) slides from each specimen were retained to investigate the pathological tissue changes on tamoxifen. The slides were studied blindly by the same investigator (Dr Alistair Williams) and this is described in more detail in a subsequent chapter.

Immunohistochemistry

From the original specimen blocks of 16 women on tamoxifen at the time of hysterectomy and 5 control women (with a history of breast cancer not on tamoxifen) 4 x 3μm tissue sections were cut and mounted onto superfrost slides to stain for markers of cellular proliferation, ER and MIB 1. Staining was performed by Miss L MacFarlane, Pathology Department, Western General Hospital, Edinburgh.

Antibody to oestrogen receptor (ER)

Sections were mounted on Superfrost slides and de-waxed in Xylene for 5 minutes twice, to dissolve all paraffin wax, followed by immersion in pure alcohol twice for 30 seconds each, followed by immersion in industrial methylated spirits for 30 seconds and then rinsed in running water. Sections were incubated in 3% hydrogen peroxide for 10 minutes to block endogenous peroxide activity (3mls Hydrogen peroxide in 100mls distilled water) and again rinsed in running water. Slides were then immersed in citrate buffer (pH 6.0) and underwent 10 minutes pressure cooker pre-treatment in a microwave for optimal antigen retrieval and were loaded onto a Shandon sequenza and placed in PBS (phosphate buffered saline) for 5 minutes.

Slides were then incubated in 100μl of Avidin/Biotin blocking kit (Vector Laboratories Ltd, Peterborough UK) for 30 minutes to block non-specific binding. 100μl per slide of primary antibody was applied (Dako ER (Dako Ltd, Ely, Cambridgeshire UK) antibody diluted in Dako background reducing diluent and Biotin form the blocking kit) and incubated at room temperature for 60 minutes. Sections were rinsed in PBS for 5 minutes.

Secondary antiserum, (Biotinylated anti-mouse IgG from Vector Laboratories) was applied to slides and incubated at room temperature for 30 minutes and then washed in PBS for 5 minutes. Sections were then incubated in StrepABComplex/horseradish peroxidase as instructed in the Duet kit for 30 minutes and rinsed in PBS.

Slides were then incubated in Dako Strept Avidin/Biotin complex reagent kit for 30 minutes at room temperature, washed in PBS and then washed in deionised water to clean the sections.

Slides were removed from the Shandon Sequenza and DAB (Diaminobenzidine) applied to each section and allowed to incubate for 5 minutes. They were washed in water and enhanced in copper solution (copper sulphate and NaCl) for a further 5 minutes staining ER positive cells brown and then counterstained in Mayers Haematoxylin for 1-2 minutes, showing non staining nuclei blue. Acid alcohol was applied to remove excess haematoxylin and weak ammonia solution (0.5% aqueous NH_3) to turn the haematoxylin from purple to blue. Slides were washed in tap water before being dehydrated by immersing in industrial methylated spirits, followed by pure alcohol x 2 for 5 minutes each, then two changes of Xylene for 5 minutes each and finally mounted in DPX (mounting medium).

MIB - 1 antibody to proliferating cells

A similar method was used for staining with MIB-1 antibody, directed against proliferating cells. The primary antibody used was MIB-1 (Biogenex, USA - British distributor, A. Menarini, Wokingham, Berkshire, UK) and slides were incubated in primary antibody for 30 minutes at room temperature. All other steps in the staining protocol were as for ER as described above.

Tamoxifen Metabolite Plasma Levels

All women had blood taken following their ultrasound scan to measure endometrial thickness for measurement of plasma tamoxifen and its metabolites 4-hydroxytamoxifen and desmethyltamoxifen. Although metabolite E is thought to be the most oestrogenic of tamoxifen's metabolites, it is present in very small amounts and was not possible to measure. 20 patients had a second blood sample taken at the time of hysteroscopy to ensure blood levels were stable and to validate the technique. Blood was placed into Heparin-Lithium vials and spun down at 3000rpm for 15 minutes to separate the plasma fraction, which was then extracted into containers, wrapped in foil (because tamoxifen is light sensitive) and frozen at (-40°C). Levels of tamoxifen and its metabolites were measured by solid phase extraction and reversed phase high performance liquid chromatography (HPLC) which was a new rapid technique developed in Edinburgh.

Materials

Standard solutions were prepared in methanol (MeOH) and stored at 4°C. MeOH and acetonitrile (ACN) were of HPLC reagent grade and triethylene (TEA) and sodium chloride (NaCI) were obtained from Sigma (Poole, UK). Water was deionised (dH_20) and bi-distilled in an Analyst HP Select still.

Plasma Preparation

Prior to extraction the samples were thawed and precipitated protein removed by centrifugation at 469g for 5 mins.

The solid phase extraction technique is shown below.

1. Activation (2ml MeOH)
2. Wash (2ml dH_2O)
3. Load sample (1ml plasma)
4. Wash (1ml dH_2O)
5. Wash (1ml dH_2O:MeOH, 50:50)
6. Wash (1ml ACN)
7. Elution (2ml MeOH:NaCl,95:5)
8. Dry under vacuum
9. Re-suspend (200µl MeOH)
10. Inject 20µl onto column

Solid phase extraction was carried out on Bond-elut C_2 3cm^3 columns. An optimum sample size of 1ml was applied to each column. Activation and pre-sample washing were carried out using 2ml volumes, after which, sample loading and post-sample washes were carried out using 1ml volumes. Final elution was with 2ml of 1M NaCl in MeOH (5:95, v/v). Eluents were dried down in a Uniscience Univap centrifuge then re-suspended in 200µl of MeOH and finally placed into 0.3ml, 8mm glass inert vials for analysis.

A second 1ml sample was extracted from each patient and to each duplicate sample 10µl Cis-tamoxifen (CIS, 0.1µg/ml) was added as an external standard to monitor extraction efficiency. At the same time 10µl of CIS was added to 190µl MeOH as a control.

1ml x 2 of plasma from an untreated patient was also applied to columns, the second 1ml sample having 10µl of mixed metabolites (0.1µg/ml) added. A second standard contained 10µl of mixed metabolites in 190µl MeOH.

High Performance Liquid Chromatography (HPLC)

The chromatography system consisted of a Merck Hitachi L-6000 solvent delivery system; a Spark-Holland Basic Marathon autosampler (set to inject 20µl); an ICT Beam Boost photochemical reaction unit; a Waters Associate Model 440 absorbance detector (set to detect 265nm) and a Hewlett-Packard HP 3394 A integrator. The stationary phase was µBondpack C_{18} packed in a 30cm x 3.9mm ID stainless steel column with a 1-cm C_{18} guard column and the mobile phase consisted of 1% TEA in MeOH. Mobile phase components were passed through a 0.22µm filter and degassed prior to use. Elution was isocratic at a flow rate of 1.2ml/min at ambient room temperature.

Method development established pH 8 as the optimal pH for resolution of tamoxifen and its metabolites. A µBondpack C_{18} column was found to give the best peak separation, symmetry and area to height ratios. The lowest signal-to-noise ratio was achieved for extraction of 1ml of plasma, with 200µl of sample loaded onto the HPLC column.

PILOT STUDY

Introduction

From the literature there appears to be a 2-3 fold increase in the incidence of endometrial cancer in women taking adjuvant tamoxifen. In view of this, should women taking tamoxifen either as adjuvant therapy or as part of ongoing breast cancer prevention trials be screened for endometrial abnormalities. Further, if screening is advocated what is the optimum screening protocol?

The aim was to establish the incidence of endometrial abnormalities in asymptomatic women on adjuvant tamoxifen and to evaluate the various screening methods available. Symptomatic women, ie those who present with abnormal bleeding/discharge are thoroughly investigated primarily to exclude malignancy and there have been various studies assessing endometrial problems in symptomatic women on tamoxifen. Establishing the incidence of abnormalities in asymptomatic women would enable conclusions to be drawn regarding screening programme requirement and which screening tool was appropriate. A further aim was to investigate the relationship between the length of time on tamoxifen and the incidence and nature of any endometrial abnormalities.

Patients and Methods

The pilot study was performed on the first 120 patients on tamoxifen recruited into the study. They all consented to have transvaginal ultrasound scan (TV USS), out-patient hysteroscopy and pipelle biopsy performed. This was to assess which was the best screening method in terms of detection of abnormalities and tolerability of the procedure.

For each patient a proforma was completed to collect data on menopausal status, age, time on tamoxifen/cumulative dose of tamoxifen, endometrial thickness on ultrasound scan, details of pipelle biopsy and hysteroscopy findings. With this information the relationship between time on tamoxifen and endometrial abnormalities could be established.

All patients were asked to complete a procedure score evaluating the pain and discomfort experienced during each procedure. This was done by means of a 10 point scale where 1=not uncomfortable and 10=very uncomfortable. This was completed after all three procedures had been performed/attempted so patients were able to compare each procedure. This was felt to be important because when evaluating a possible screening tool the tolerability of each procedure needs to be established.

Results

Table 1 gives patient details and results from endometrial screening for the first 120 women on tamoxifen.

Of the 120 patients (age range 40-76) in the pilot study (18 premenopausal and 102 postmenopausal) 66 had an endometrium measuring ≤5mm or were within the accepted cycle limits for premenopausal women (anything up to 10mm in the late secretory phase is normal) and 54 had thickened endometrium as measured by transvaginal ultrasound scan. The first 10 women with thickened endometrium on ultrasound scan had the scan repeated by a single consultant radiologist to confirm endometrial thickness and in all cases measurements were comparable.

107 women went on to have out-patient hysteroscopy. At hysteroscopy, all women with normal thickness endometrium on scan had normal or atrophic endometrium. There were 2 women whose endometrium measured 4mm on scan who, although had a normal endometrium at hysteroscopy, were found to have other abnormalities, one being a tiny polyp at the internal cervical os and the other a small submucous fibroid.

13 women could not be hysteroscoped. This was because of cervical stenosis in 7 women, a large cervical polyp in 1 woman, being unable to pass and open the speculum sufficiently to view the cervix in 3 women and 1 refusal to have hysteroscopy; this latter woman agreed initially to have all three procedures performed as part of the pilot study but, following a normal scan, she declined any further investigation. One woman was not deemed fit enough to have hysteroscopy performed as she became very breathless when placed flat at the outset of the procedure.

Only 4 women who had a failed hysteroscopy had thickened endometrium on TV USS. All 4 went on to have a D & C to investigate this further; 3 were found to have atrophic endometrium and the other had a benign polyp at D & C.

The remaining 9 women who had a failed hysteroscopy did not have thickened endometrium on scan and had no further investigation performed. Each individual woman

was discussed with a consultant gynaecologist who agreed that subjecting these asymptomatic women to D & C was not justified.

Of the 50 women who were identified on TV USS to have thickened endometrium and went on to have hysteroscopy performed, 19 actually had atrophic/normal endometrium at hysteroscopy and 31 had benign endometrial changes.

These benign changes were:

polyp	13
cystic endometrium	4
oedematous endometrium	13
submucous fibroid	1

This gives a 38% false positive scan rate (19/50) and a 26% (31/120) incidence of benign changes although only 45% (14/31) of these benign changes are due to polyps and fibroids, the remainder being cystic or oedematous endometrium.

Pipelle was attempted in all 120 women but was successful in only 49 (41%). Of those 49, tissue was obtained in only 5, all of which were benign.

Of the 18 premenopausal women, pipelle was unsuccessful in 8 (44%), was successful but no tissue was obtained in 8/18 (44%) and was successful and benign tissue obtained in 2/18 (11%). Similarly, in the 89 postmenopausal women (the 13 women where hysteroscopy was also unsuccessful have been excluded from this calculation) pipelle was not successful in 40/89 (45%), was successful but no tissue was obtained in 36/89 (40%) and was successful and benign tissue obtained in 3/89 (<5%).

Table 2 gives all patient details of time on tamoxifen and cumulative dose and endometrial thickness on ultrasound scan.

Time on tamoxifen was calculated in months.

Cumulative dose was calculated by:

Dose per month = 365 x 20mg/12 = 610mg
Cumulative does = dose per month (610mg) x time on tamoxifen in months

In one patient this calculation was not used because she did not take tamoxifen regularly. Her cumulative dose was adjusted accordingly.

There was a highly significant positive correlation between length of time on tamoxifen (and cumulative dose of tamoxifen) and endometrial thickness as measured using transvaginal ultrasound scan, p=0.0003 on Spearman correlation.

The relationship between endometrial thickness and duration of tamoxifen use is demonstrated graphically in Figures 1 and 2 and the relationship between endometrial thickness and cumulative dose of tamoxifen in Figures 3 and 4.

Table 1

Endometrial screening details for the first 120 women on tamoxifen.

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy	Pipelle
1. EF	74	post	17	N/atrophic	No
2. HM	56	post	6	oedematous	No
3. SP	67	post	7	N/atrophic	Yes-NT
4. OK	61	post	20	N/atrophic	No
5. CC	49	pre	3	N/atrophic	Yes-NT
6. AC	53	post	4	N/atrophic	Yes-NT
7. EB	52	pre	2	N/atrophic	Yes-B
8. MC	67	post	4	N/atrophic	No
9. BU	45	pre	5	N/atrophic	No
10. EH	61	post	3	N/atrophic	No
11. JB	65	post	7	cystic	Yes-NT
12. AS	61	post	3	N/atrophic	No
13. MS	67	post	3	N/atrophic	Yes-NT
14. EB	69	post	38	oedematous	No
15. JA	64	post	23	polyp	Yes-NT
16. MM	52	pre	15	cystic	No
17. MW	61	post	12	oedematous	No
18. MMc	66	post	5	N/atrophic	No
19. EM	45	post	2	N/atrophic	No
20. MC	67	post	5	N/atrophic	Yes-NT
21. MS	70	post	22	oedematous+polyp	No
22. CM	64	post	2	not done cervical stenosis	No
23. GK	50	post	6	N/atrophic	No
24. EB	62	post	3	N/atrophic	No

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy	Pipelle
25. MG	42	pre	5	N/atrophic	No
26. HD	66	post	4	N/atrophic	Yes-NT
27. MM	62	post	4	N/atrophic	No
28. WK	45	post	3	N/atrophic	Yes-NT
29. MK	70	post	3	N/atrophic	No
30. MH	65	post	7	N/atrophic	No
31. KR	58	post	11	N/atrophic	Yes-NT
32. LR	67	post	8	N/atrophic	No
33. ER	55	post	5	N/atrophic	Yes-NT
34. JR	68	post	2	N/atrophic	No
35. CS	53	post	7	submucous fibroid	Yes-NT
36. SR	45	pre	3	N/atrophic	Yes-NT
37. MR	64	post	4	N/atrophic	No
38. JU	54	post	14	cystic submucous fibroid	No
39. ED	60	post	7	N/atrophic	No
40. AD	72	post	3	N/atrophic	No
41. MM	67	post	3	N/atrophic	Yes-NT
42. AS	75	post	2	N/atrophic	No
43. AMc	68	post	6	N/atrophic	No
44. CH	65	post	3	N/atrophic	No
45. SD	72	post	6	polyp	Yes-NT
46. JA	74	post	4	N/atrophic (polyp)	Yes-NT
47. GT	69	post	3	N/atrophic	No
48. LL	66	post	5	not done; unable to pass speculum	No
49. KD	73	post	6	N/atrophic	No
50. SM	61	post	4	N/atrophic	No
51. IN	51	post	3	N/atrophic	Yes-NT

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy	Pipelle
52. NB	66	post	6	oedematous	No
53. MM	74	post	7	N/atrophic	Yes-NT
54. DS	52	pre	5	N/atrophic	Yes-NT
55. JR	54	post	20	oedematous	Yes-NT
56. ES	64	post	13	not done cervical stenosis	No
57. MG	66	post	27	oedematous+polyp	No
58. MD	59	post	3	N/atrophic	No
59. MP	42	pre	7	N/atrophic	Yes-NT
60. FB	71	post	13	cystic	No
61. MR	54	pre	25	oedematous+cystic	Yes-NT
62. ES	67	post	16	oedematous+polyp	No
63. CA	65	post	19	oedematous+polyp	No
64. PC	48	pre	4	N/atrophic	Yes-NT
65. IH	74	post	3	N/atrophic	No
66. FN	75	post	15	N/atrophic	Yes-NT
67. EF	67	post	3	N/atrophic	No
68. AL	76	post	5	N/atrophic	Yes-NT
69. JC	52	pre	12	polyp	No
70. DM	58	post	15	polyp	No
71. FR	49	post	3	N/atrophic	Yes-NT
72. JD	57	post	7	N/atrophic	Yes-B
73. JL	64	post	9	not done cervical stenosis	No
74. IR	54	post	4	N/atrophic	Yes-NT
75. ED	62	post	6	N/atrophic	Yes-B
76. JC	65	post	10	oedematous	Yes-NT
77. MD	65	post	8	oedematous	Yes-NT
78. WP	62	post	9	N/atrophic	No

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy	Pipelle
79. MT	62	post	6	N/atrophic	No
80. DS	64	post	3	N/atrophic	No
81. MH	55	post	15	N/atrophic	Yes-NT
82. EH	64	post	5	N/atrophic	Yes-NT
83. DR	80	post	6	oedematous	No
84. ER	67	post	4	not done - not fit for procedure	No
85. KM	54	pre	6	N/atrophic submucous fibroid	No
86. JB	50	pre	4	N/atrophic	No
87. DK	76	post	11	polyp	No
88. EG	68	post	4	N/atrophic	No
89. EC	67	post	6	polyp	No
90. AS	59	post	7	N/atrophic	Yes-NT
91. BR	56	pre	4	N/atrophic	Yes-NT
92. GC	59	post	20	N/atrophic	Yes-NT
93. CR	60	post	2	N/atrophic	Yes-NT
94. AM	65	post	3	not done cervical stenosis	No
95. MM	71	post	3	not done - unable to pass speculum	No
96. JD	40	pre	4	N/atrophic	No
97. AB	48	post	3	N/atrophic	Yes-B
98. JD	47	post	5	not done declined procedure	Declined
99. AC	63	post	4	N/atrophic submucous fibroid	Yes-NT
100. JB	52	pre	11	polyp	Yes-B
101. JH	63	post	19	polyp	Yes-NT
102. MH	64	post	30	polyp	No

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy	Pipelle
103. AG	50	post	2	N/atrophic	Yes-NT
104. AF	79	post	9	not done	No
105. IM	73	post	3	N/atrophic	Yes-NT
106. IM	47	pre	14	polyp	Yes-NT
107. RM	61	post	5	not done cervical stenosis	No
108. AD	57	post	1	N/atrophic	Yes-NT
109. HN	74	post	4	not done - unable to pass speculum	No
110. CS	59	post	20	not done cervical polyp	No
111. AS	52	post	3	N/atrophic	Yes-NT
112. AS	42	pre	4	N/atrophic	No
113. CT	56	post	4	not done cervical stenosis	No
114. EW	70	post	12	polyp+cystic	No
115. WW	58	post	3	N/atrophic	No
116. IT	50	post	6	polyp	No
117. SC	66	post	3	N/atrophic	No
118. EC	65	post	3	N/atrophic	No
119. ML	58	post	11	polyp	Yes-NT
120. JM	59	post	5	N/atrophic	Yes-NT

Pipelle biopsy:

A 'No' response indicates that the procedure was attempted. Reasons for failure were:

1. cervix not visualised.

2. unable to pass the pipelle through the cervical os

NT = no tissue obtained

B = benign

Hysteroscopy:

N = normal

Table 2

Patient details regarding length of time on tamoxifen, cumulative dose of tamoxifen and endometrial thickness as assessed by TV USS for the first 120 patients.

Patient No	Time on Tamoxifen (mths)	Cumulative Dose Tamoxifen (mg)	Endometrial thickness-TV USS (mm)
1. EF	185	112850	12
2. HM	77	46970	6
3. SP	61	37210	7
4. OK	57	34770	20
5. CC	30	18300	3
6. AC	14	8540	4
7. EB	15	9150	2
8. MC	70	42700	4
9. BU	108	65880	5
10. EH	69	42090	3
11. JB	62	37820	7
12. AS	48	29280	3
13. MS	74	45140	3
14. EB	75	45750	38
15. JA	80	48800	23
16. MM	120	73200	15
17. MW	86	52460	12
18. MM	95	57950	5
19. EM	65	39650	2
20. MC	36	21960	5
21. MS	97	59170	22
22. CM	72	43920	2
23. GK	36	21960	6
24. EB	61	37210	3

Patient No	Time on Tamoxifen (mths)	Cumulative Dose Tamoxifen (mg)	Endometrial thickness-TV USS (mm)
25. MG	24	14640	5
26. HD	17	10370	4
27. MM	36	21960	4
28. WK	32	19520	3
29. MK	111	67710	3
30. MH	83	50630	7
31. KR	96	58560	11
32. LR	31	18910	8
33. ER	66	40260	5
34. JR	26	15860	2
35. CS	26	15860	7
36. SR	25	15250	3
37. MR	51	31110	4
38. JU	38	23180	14
39. ED	24	14640	7
40. AD	134	81740	3
41. MM	36	21960	3
42. AS	104	63440	2
43. AM	34	20740	6
44. CH	48	29280	3
45. SD	79	48190	6
46. JA	98	59780	4
47. GT	49	29890	3
48. LL	23	14030	5
49. KD	22	13420	6
50. SM	61	37210	4
51. IN	5	3050	3

Patient No	Time on Tamoxifen (mths)	Cumulative Dose Tamoxifen (mg)	Endometrial thickness-TV USS (mm)
52. NB	62	37820	6
53. MM	66	40260	7
54. DS	96	58560	5
55. JR	190	115900	20
56. ES	37	22570	13
57. MG	153	93330	27
58. MD	74	45140	3
59. MP	14	8540	7
60. FB	62	37820	13
61. MR	86	52460	25
62. ES	86	52460	16
63. CA	124	75640	19
64. PC	12	7300	4
65. IH	24	14640	3
66. FN	84	51240	15
67. EF	37	22570	3
68. AL	159	96990	5
69. JC	84	51240	12
70. DM	81	49410	15
71. FR	60	36600	3
72. JD	10	6100	7
73. JL	16	9760	9
74. IR	20	12200	4
75. ED	67	40870	6
76. JC	61	37210	10
77. MD	62	37820	8
78. WP	62	37820	9

Patient No	Time on Tamoxifen (mths)	Cumulative Dose Tamoxifen (mg)	Endometrial thickness-TV USS (mm)
79. MT	180	109800	6
80. DS	97	59170	3
81. MH	49	29890	15
82. EH	60	36600	5
83. DR	73	44530	6
84. ER	30	18300	4
85. KM	5	3050	6
86. JB	38	23180	4
87. DK	120	73200	11
88. EG	72	43920	4
89. EC	49	29890	6
90. AS	133	81130	7
91. BR	60	36600	4
92. GC	73	44530	20
93. CR	61	37210	2
94. AM	89	54290	3
95. MM	51	31110	3
96. JD	26	15860	4
97. AB	15	9150	3
98. JD	30	18300	5
99. AC	71	43310	4
100. JB	76	46360	11
101. JH	37	22570	19
102. MH	84	51240	30
103. AG	24	14640	2
104. AF	65	39650	9
105. IM	13	7930	3

Patient No	Time on Tamoxifen (mths)	Cumulative Dose Tamoxifen (mg)	Endometrial thickness-TV USS (mm)
106. IM	36	21960	14
107. RM	43	26230	6
108. AD	49	29890	1
109. HN	72	43920	4
110. CS	61	37210	20
111. AS	11	6700 *	3
112. AS	82	50020	4
113. CT	133	81130	4
114. EW	70	42700	12
115. WW	25	15250	3
116. IT	84	51240	6
117. SC	16	9760	3
118. EC	51	31110	3
119. ML	57	34770	11
120. JM	47	28670	5

* Does not take tamoxifen regularly therefore smaller cumulative dose.

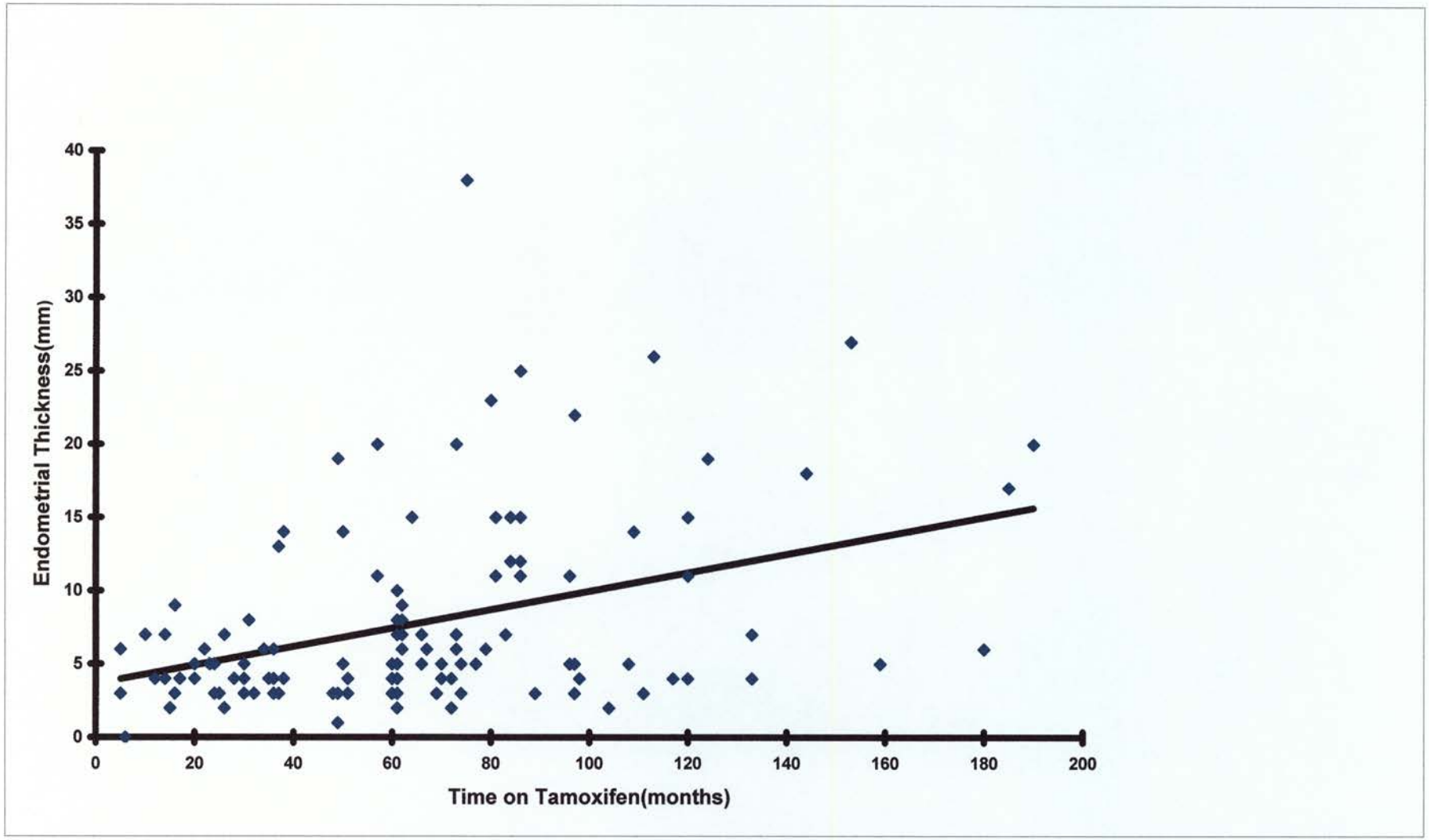


Figure 1
Relationship between endometrial thickness and tamoxifen duration.

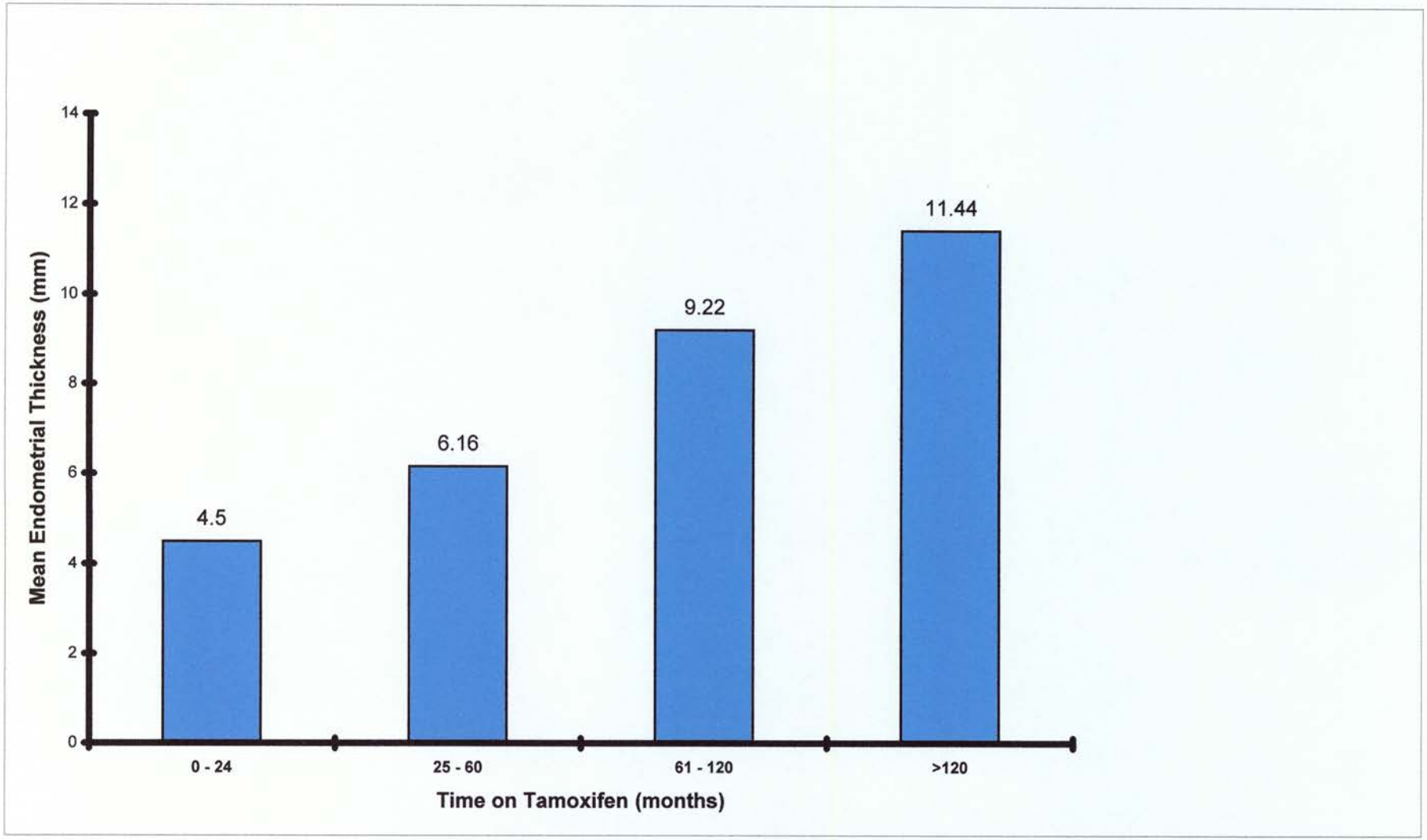


Figure 2
Relationship between endometrial thickness and duration of tamoxifen use.

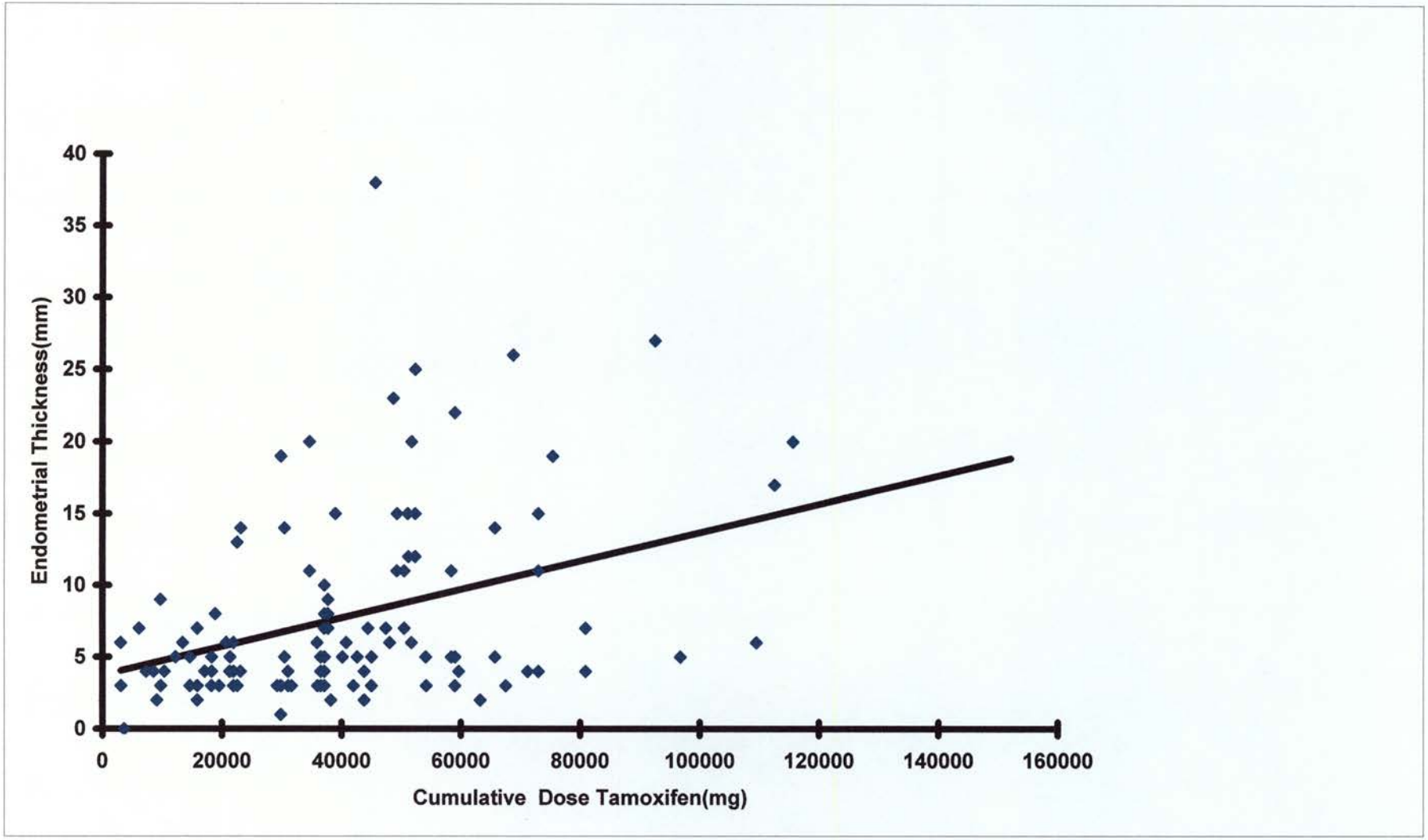


Figure 3
Relationship between endometrial thickness and cumulative dose of tamoxifen.

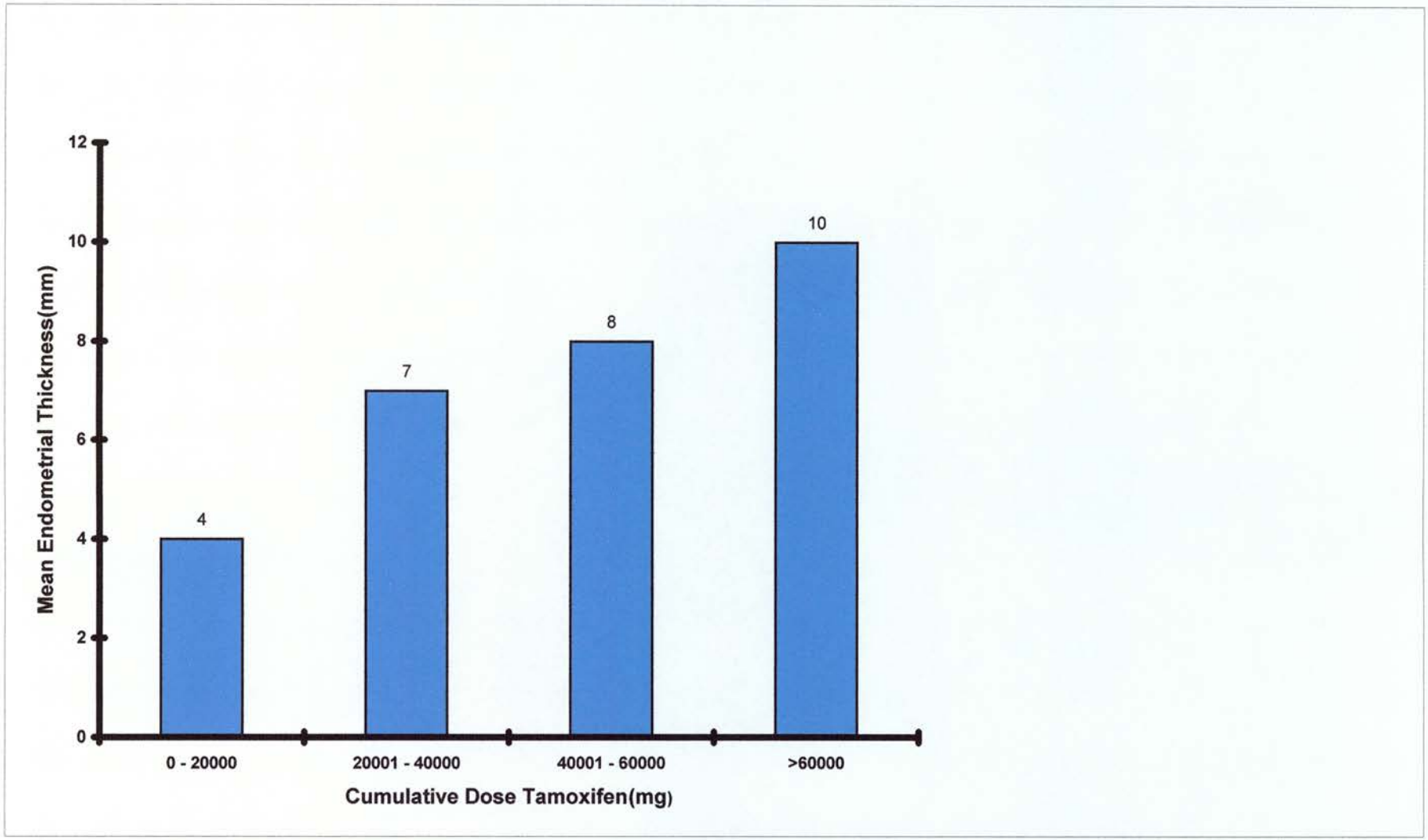


Figure 4
Relationship between mean endometrial thickness and cumulative dose of tamoxifen.

Table 3 shows the results from the procedure scores completed by each patient following all three procedures.

13 patients did not complete all three procedure scores. This was because in 10 of the women hysteroscopy was not possible and they were not asked to complete the three procedure scores. One woman forgot to complete the scores and the remaining 2 were the women who either declined to have hysteroscopy or were deemed not fit enough to have the procedure performed. A further 5 patients did not score the pipelle biopsy because they were in the group where pipelle was unsuccessful and therefore felt unable to score the procedure. One woman did not score the hysteroscopy as it was not performed because of cervical stenosis but she completed the other two scores.

All scores are summarised below.

Score	TV USS	Hysteroscopy	Pipelle
1	59	27	30
2	30	21	28
3	14	21	20
4	4	11	14
5	-	14	6
6	-	4	2
7	-	2	-
8	-	6	-
9	-	-	1
10	-	-	1

This summary table is demonstrated graphically in Figure 5

All patients scored the pain/discomfort associated with TV USS 4/10 or less (Fig 6) 75% of patients scored the pain/discomfort during hysteroscopy 4/10 or less (Fig 7) and 90% scored pipelle 4/10 or less (Fig 8).

From this analysis, TV USS was statistically better tolerated than out-patient hysteroscopy or pipelle biopsy ($P<0.0001$, Mann Whitney U test). There was no statistical difference between hysteroscopy and pipelle biopsy ($p = 0.0607$, Mann Whitney U test).

Table 3

Procedure scores for first 120 women on tamoxifen.

Patient No	TV USS	Hyster-oscopy	Pipelle
1.	2	4	4
2.	1	1	1
3.	1	1	1
4.	1	4	2
5.	2	2	2
6.	1	2	2
7.	1	3	5
8.	1	1	1
9.	1	6	1
10.	1	1	1
11.	4	4	4
12.	2	4	2
13.	2	5	4
14.	1	1	1
15.	2	1	6
16.	1	2	2
17.	2	2	2
18.	2	1	2
19.	1	3	3
20.	1	2	1
21.	1	1	1
22.	1	-	1
23.	1	2	3
47.	1	1	-

Patient No	TV USS	Hyster-oscopy	Pipelle
24.	1	3	1
25.	3	4	-
26.	1	1	5
27.	3	6	4
28.	2	3	2
29.	1	5	3
30.	1	2	1
31.	1	1	3
32.	3	3	4
33.	2	5	2
34.	1	1	-
35.	3	4	3
36.	1	6	4
37.	2	1	2
38.	1	3	1
39.	1	1	-
40.	1	2	1
41.	3	3	3
42.	2	3	2
43.	1	1	1
44.	3	6	4
45.	1	1	1
46.	1	3	2
74.	1	1	1

Patient No	TV USS	Hyster-oscopy	Pipelle
48.			
49.	2	8	3
50.	1	2	2
51.	1	1	1
52.	2	2	2
53.	2	2	2
54.	3	2	3
55.	1	4	1
56.			
57.	3	4	2
58.	1	3	1
59.	4	8	4
60.	2	1	2
61.	2	2	2
62.	2	5	3
63.	1	5	1
64.	3	1	3
65.	3	3	3
66.	2	5	2
67.	1	3	6
68.	2	4	3
69.	2	2	2
70.	3	8	5
71.	2	5	4
72.	1	2	3
73.			
101.	1	1	1
102.	1	5	2

Patient No	TV USS	Hyster-oscopy	Pipelle
75.	1	3	2
76.	3	3	5
77.	2	2	2
78.	2	1	3
79.	1	5	5
80.	2	5	3
81.	1	3	1
82.	1	2	1
83.	1	3	2
84.			
85.	4	5	4
86.	2	4	4
87.	1	1	2
88.	4	5	4
89.	1	1	1
90.	2	2	2
91.	3	5	3
92.	2	3	9
93.	3	7	3
94.			
95.			
96.	1	3	5
97.	1	1	1
98.			
99.	1	8	1
100.	1	8	3
111.	1	3	2
112.	1	1	1

Patient No	TV USS	Hyster-oscopy	Pipelle
103.	1	2	1
104.			
105.	1	1	1
106.	1	2	4
107.			
108.	1	3	4
109.			
110.			

Patient No	TV USS	Hyster-oscopy	Pipelle
113.			
114.			
115.	1	7	10
116.	1	5	3
117.	1	2	1
118.	2	8	-
119.	1	3	1
120.	2	4	2

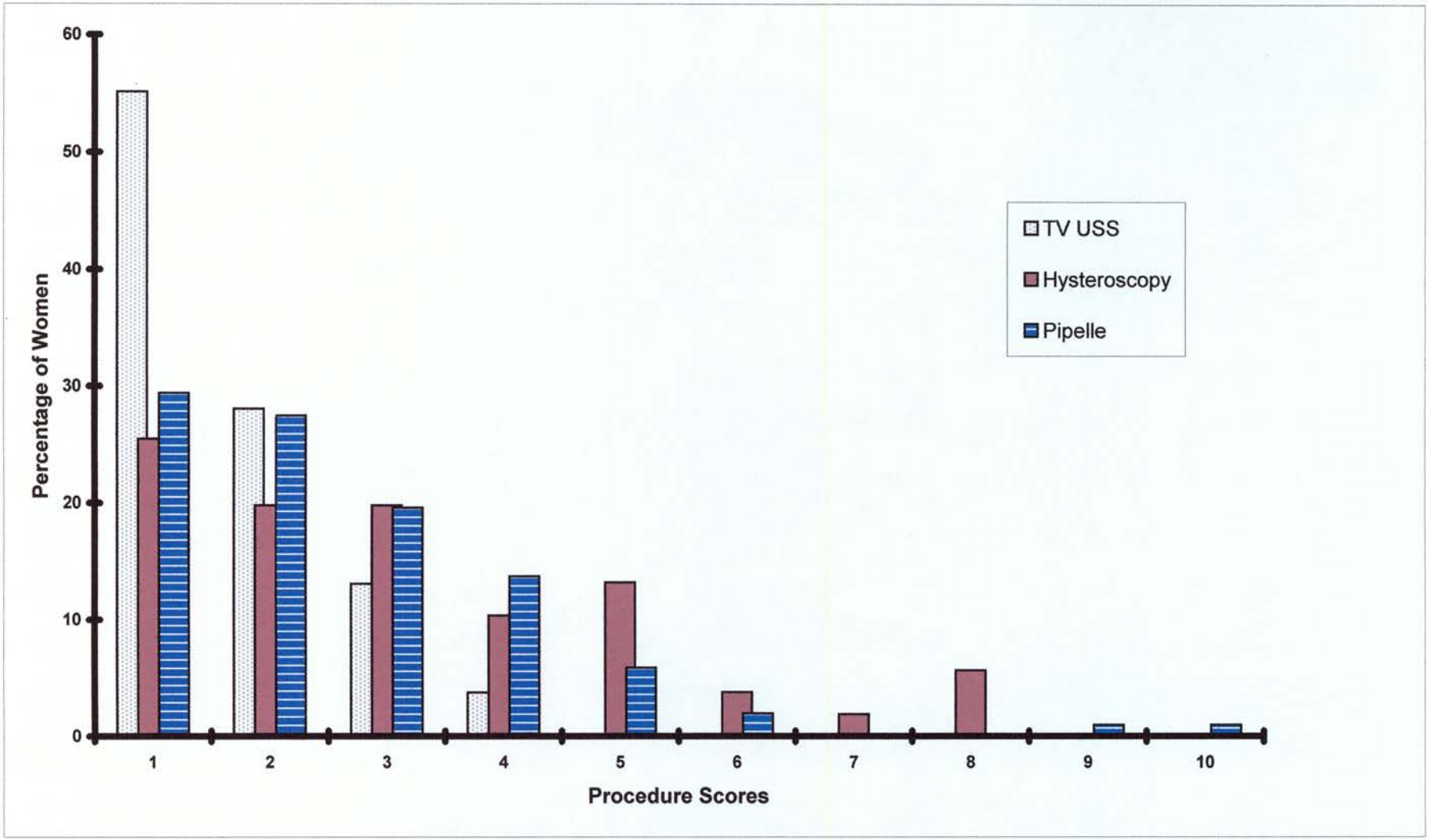


Figure 5
Summary of procedure scores.

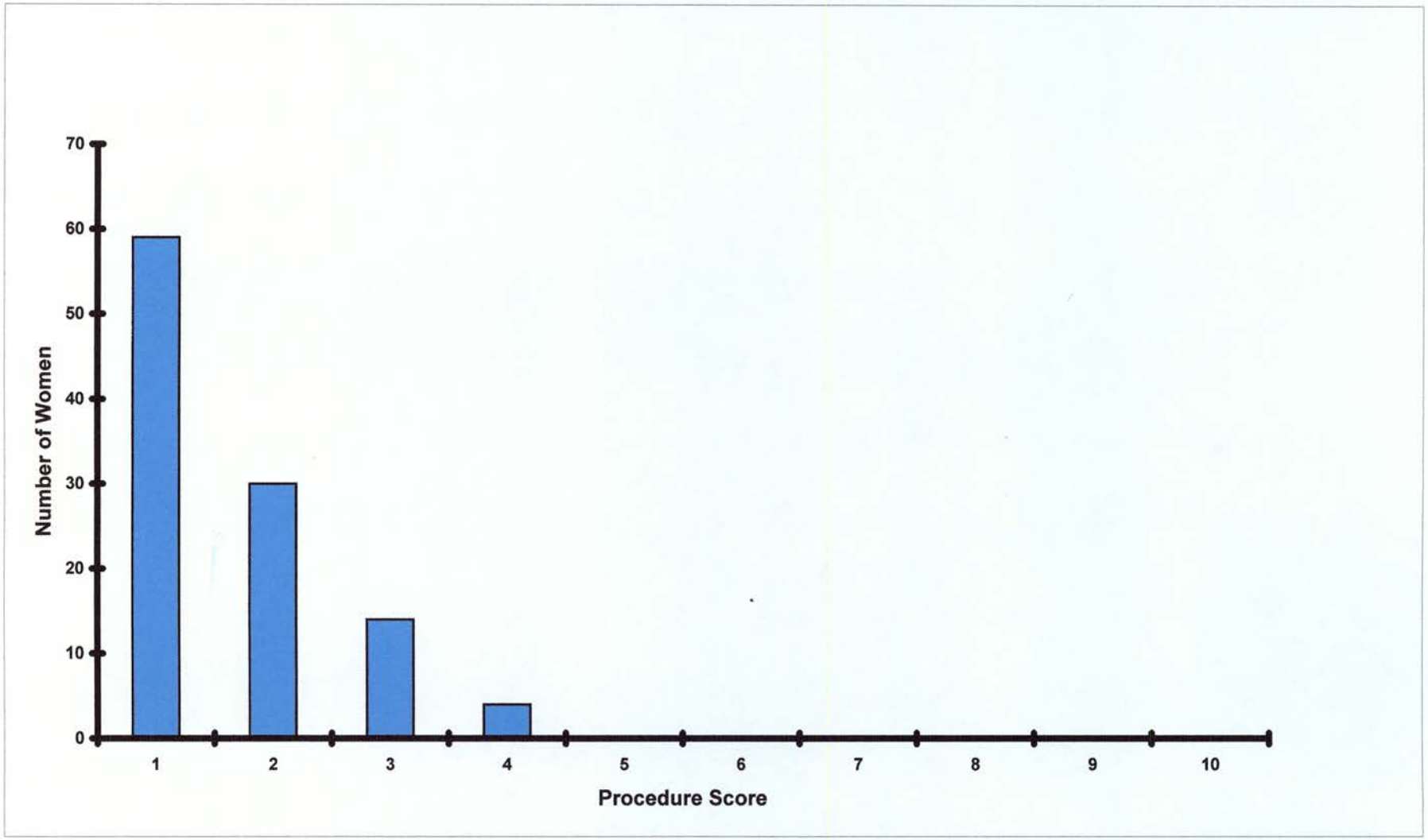


Figure 6
Procedure score for TV USS.

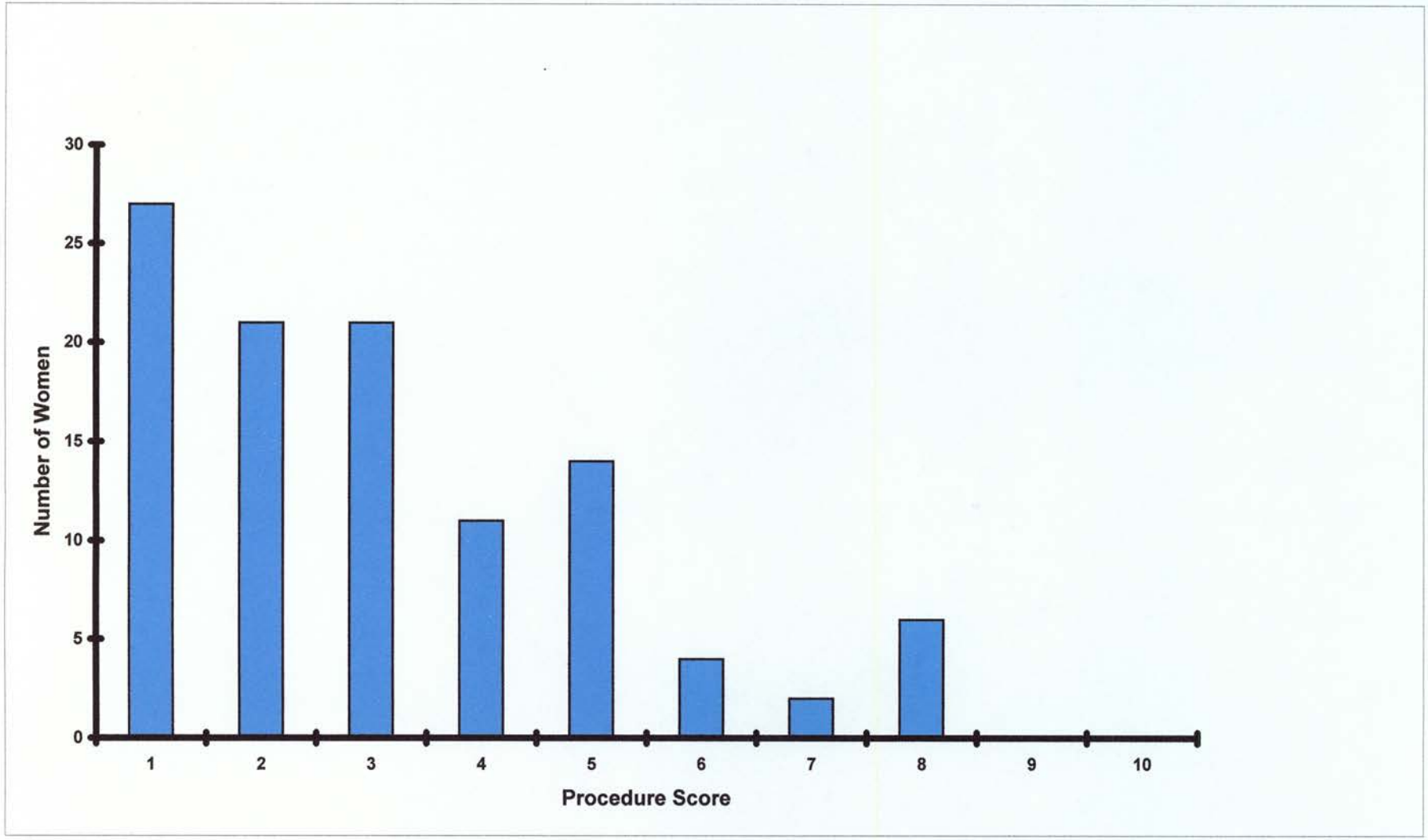


Figure 7
Procedure score for hysteroscopy.

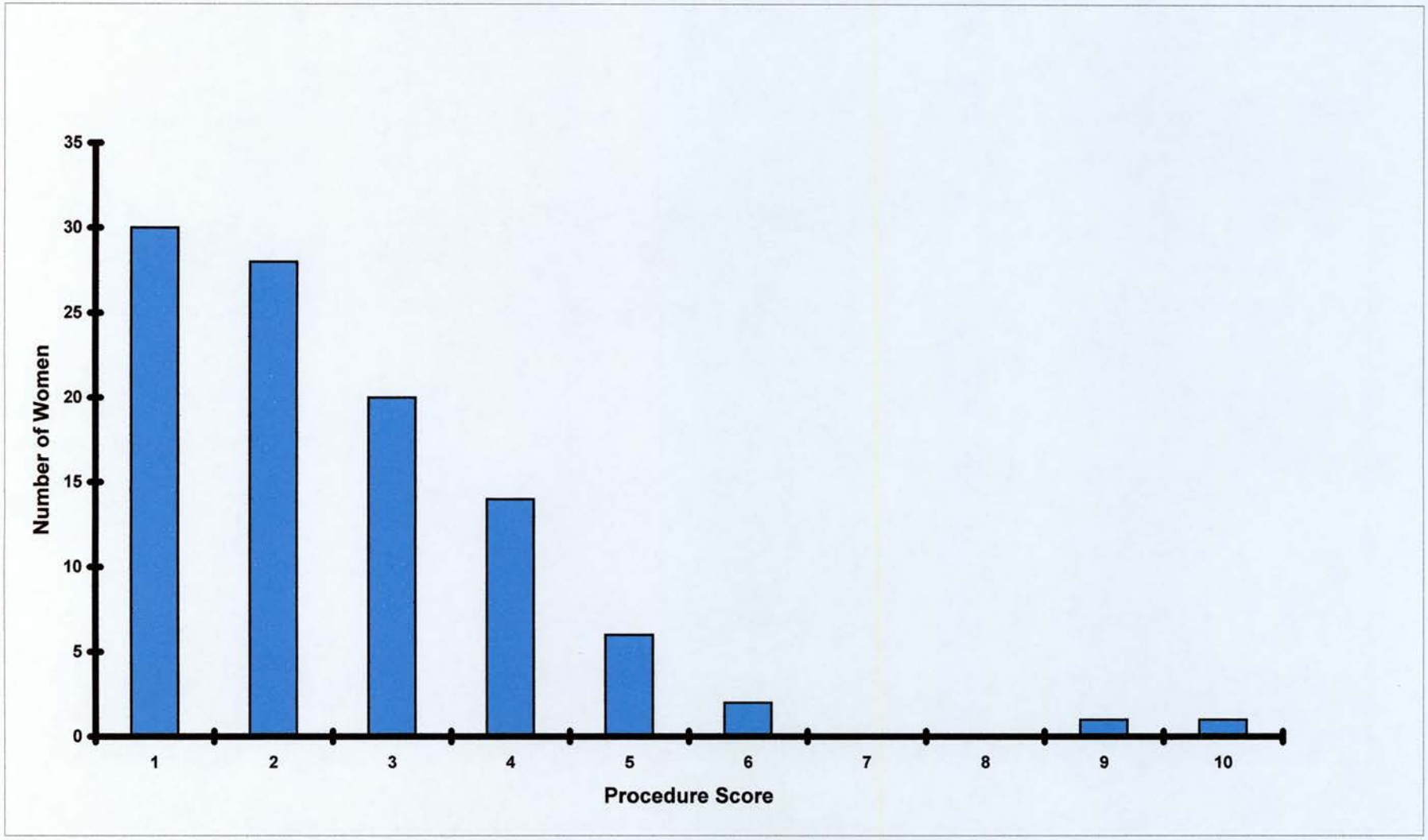


Figure 8
Procedure score for pipelle biopsy.

Discussion

One of the main aims of the pilot study was to establish the best algorithm for screening asymptomatic women on tamoxifen with respect to detection of abnormalities. Once this was established, the remaining women recruited into the study would be offered the best screening test.

When implementing a screening programme, the WHO recommends that the test performed should be:

1. Acceptable to patients.
2. Valid

[Wilson 1968]

Validity of a test involves repeatability and accuracy (high sensitivity and specificity, a high positive predictive value and a low negative value).

This can be represented in the following table:

Screening Test	Reference Test		
	+ve	-ve	
+ve	true +ve (a)	false +ve (b)	total +ve by screening (a + b)
-ve	false -ve (c)	true -ve (d)	total -ve by screening (c+d)
	total true +ve (a+c)	total true -ve (b+d)	grand total (a+b+c+d)

Transvaginal ultrasound scan met the majority of these criteria. The procedure scores completed by each patient confirmed that ultrasound scanning was significantly better tolerated than hysteroscopy or pipelle biopsy ($p<0.0001$). This was not surprising because of the less invasive nature of the technique making it more tolerable. There were no significant side effects reported from having transvaginal ultrasound scanning performed.

Ultrasound also proved to be repeatable and accurate, ie valid. The first 10 women with thickened endometrium on USS had the scan repeated by a consultant radiologist to confirm endometrial thickness. In all cases the measurements were comparable. Thereafter only in cases where the scan was unclear was it repeated by another operator (a consultant radiologist with specific expertise).

Ultrasound scanning also proved to be highly sensitive as all endometrial abnormalities seen at hysteroscopy were detected on ultrasound scan as thickened endometrium. There were 2 false negative results - both in patients who had endometrium measuring 4mm on ultrasound scan with one having a tiny polyp at the internal os and the other a small submucous fibroid. This is in keeping with other studies reporting an ultrasound sensitivity of 54-97% [Alcazar 1996, Cacciatore 1994, Dijkhuizen 1996, Haller 1996, Towbin 1996, Langer 1997].

However, TV USS did not have a high specificity because there was a high incidence of false positive scan findings, ie 45% (54/120) of women who had thickened endometrium on ultrasound had no significant abnormalities at hysteroscopy. 38% of these women had no abnormality at hysteroscopy (atrophic/normal endometrium) and 31% (17/54) had only oedematous or cystic endometrium. The pathology detected was all benign, not requiring any further treatment. The specificity achieved in the pilot study was 66% (70/106) and is comparable with other studies where it has been reported as ranging from 48-90%, although these studies were not investigating women on tamoxifen [Alcazar 1996, Cacciatore 1994, Dijkhuizen 1996, Haller 1996, Towbin 1996, Cohen 1993(b), Langer 1997]. The presence of thickened endometrium on USS and normal endometrium at hysteroscopy has been reported although the pathology accounting for this remains unclear [Marconi 1997, Anteby 1992, Bornstein 1994, Touraine 1995].

It became increasingly clear when performing the study that the longer women had been on tamoxifen the more likely it was to find thickened endometrium on TV USS. In the pilot study this relationship was highly significant ($p<0.0001$). This has implications for screening because the longer women are on tamoxifen the less specific the screening tool becomes. The reason for this apparent endometrial thickening on USS is a matter of debate. It has been postulated that the thickening is due to endometrial serosal or superficial myometrial oedema and presumably the longer women are under the stimulus of tamoxifen the more likely this is to occur. Although this relationship is highly significant it is not uniform, ie patient No 29 was on tamoxifen for 111 months and had an endometrium measuring 3mm yet patient No 56 took tamoxifen for 37 months and had an endometrium measuring 13mm. From the pilot study there does not appear to be any accurate way of predicting precisely who will have thickened endometrium on TV USS and time on tamoxifen alone is not 100% predictive.

Similarly, where there was thickened endometrium on ultrasound, it was not possible to predict those women who had atrophic/normal endometrium and those with benign changes on hysteroscopy.

It would be desirable for TV USS to be more specific if it is to be used as a screening tool. One way to do this would be to increase the abnormal cut off in postmenopausal women to 8mm from 5mm. This would reduce the number of false positive scans but attempts to make tests more specific usually make them less sensitive. There is no scientific basis for selecting an 8mm cut off and, as the mechanism behind this apparent thickening is unknown and there is marked inter-patient variability, thus it was considered most sensible to persist with the 5mm cut off which is currently used to assess postmenopausal endometrium in the general population as it has been shown to accurately identify those with endometrial abnormalities.

Transvaginal ultrasound has a number of advantages in that it is simple, quick, safe and relatively cheap. Although it requires dedicated equipment and training, once a machine is purchased and staff are trained, the cost per scan is minimal. It also has the advantage that the ovaries can be screened at the same time to exclude ovarian pathology. Although there is no evidence that tamoxifen exerts any effect on the ovaries, ovarian cancer is more

common in older women. However, the high false positive rate of this test leads to undesirable effects in terms of increasing patient anxiety because they require a second investigation to exclude a serious underlying abnormality.

Hysteroscopy therefore could be considered as a more appropriate screening tool. It allows direct vision of the endometrial cavity and directed biopsy and is regarded by many as the 'gold standard' investigation [Gimpelson 1995, Finikiotis 1989, Gupta 1996, Parasnis 1992, Towbin 1996]. Studies have reported a high sensitivity (79-100%) and a high specificity (93-100%) [Alcazar 1996, Cacciatore 1994, Haller 1996, Towbin 1996, Torrejon 1997]. However, a screening test should be cheap, simple, quick and acceptable to patients and hysteroscopy does not fulfil these criteria [Indman 1995, Karlsson 1994]. It is more invasive than TV USS and significantly more uncomfortable for the patient ($p<0.0001$) as assessed in the current study. Most studies, when attempting to reduce the discomfort experienced, have used paracervical block [Cooper 1995, Towbin 1996] but on questioning women in the present study, the discomfort experienced appeared to be related to insufflation of the uterine cavity with CO_2 rather than from passing the instrument through the cervical os (even in women where a small degree of cervical dilation was required). Of interest, the pain experienced was similar in nature to that experienced when performing the pipelle biopsy and an analysis of procedure scores demonstrated no significant difference in discomfort experienced between hysteroscopy and pipelle biopsy. Other studies have also shown out-patient hysteroscopy to be well tolerated [Nagele 1996, Cooper 1995, Bradley 1995]. This would surprise most gynaecologists who believe pipelle to be a relatively painless procedure.

Hysteroscopy is more expensive to perform than ultrasound scan. It requires more specialised equipment and the cost per procedure is greater because equipment needs to be sterilised between patients and nursing assistance is necessary. Consequently it takes longer to perform (approximately 30 minutes per patient for hysteroscopy versus approximately 20 minutes for TV USS). Whilst one assistant is essential to help with the equipment, two are desirable, the second present mainly to distract and monitor the patient during the procedure. Once established it is relatively quick and simple to perform and was more acceptable to patients than anticipated which may be because the procedure was not only explained fully to patients beforehand but they received an information leaflet to read

at home prior to the procedure. Hysteroscopy does require specialised training to become proficient in the technique and expertise in dealing with any side effects experienced, eg cervical shock is also required.

As hysteroscopy allows direct vision of the whole endometrial cavity abnormalities, particularly polyps can be clearly visualised and any biopsies which are performed can be targeted. This gives an advantage over both ultrasound scanning and pipelle as any abnormality detected on ultrasound scanning cannot usually be identified as anything other than thickened endometrium. Pipelle biopsy is a blind procedure and as such may miss focal lesions and abnormalities such as polyps.

The cystic endometrium seen in 4 women in the pilot study has been previously described with tamoxifen therapy [Hulka 1993, Goldstein 1994, Cohen 1996(a), Ciatto 1994, Anteby 1992] but oedematous endometrium has not. This interesting finding would be in keeping with the theory of tamoxifen causes serosal or superficial myometrial oedema explaining why attempts at biopsying this were unsuccessful, suggesting that the pathology does not lie in the endometrium, otherwise biopsy should have been possible. Marconi [1997] and others have also suggested that at hysteroscopy biopsy of thickened endometrium seen on ultrasound scanning is frequently unsuccessful at obtaining material but does not offer an explanation for this [Touraine 1995].

There were a number of women who did not have hysteroscopy performed. This was mainly because of cervical stenosis making it impossible to pass the hysteroscope through the cervical os. Cervical stenosis is more common in nulliparous women and postmenopausal women. In the presence of thickened endometrium in these women, further invasive investigation in the form of D & C is required. Of the 4 women in the pilot study who went on to D & C, 3 had atrophic endometrium and 1 had a polyp. All of these women had endometrium measuring >8mm so even if the ultrasound upper limit of normal was increased from 5mm to 8mm, these women would still have been subjected to invasive procedures. It could be that paracervical block in these women may have allowed sufficient dilatation of the cervical os to allow the procedure to have been performed as an out-patient. Therefore, although hysteroscopy is an excellent investigative tool, it does have limitations when being considered as a screening test.

The third test investigated in the pilot group was pipelle biopsy. The pipelle du Cornier is a device which has been in use since 1984 [Cornier 1984] and has become standard practise for the out-patient investigation of abnormal uterine bleeding/discharge. It is relatively easy to perform in the out-patient setting, is quick and cheap and acceptable to patients and therefore appears to be a potential screening tool [Eddowes 1990]. However, the results obtained from the pilot study do not support its use as a screening tool. Although pipelle was attempted in all women, it was only successful in 49 (41%) and of those 49 sufficient tissue was obtained for pathological assessment in only 5, all of which were benign. This is a very poor success rate, ie ability to pass the pipelle into the endometrial cavity, and is not supported by most current literature which reports a 5-10% failure rate, although this is higher in postmenopausal women (15-25%) [Batool 1994, Reid 1993]. The literature reports failure to obtain an adequate sample in 20-30% of women [Gupta 1996, Guisa-Chiferi 1996, Weber 1997] with higher rates in postmenopausal women. Batool [1994] in his study of 70 postmenopausal women with bleeding reported that in only 13/70 was there an adequate sample for diagnostic purposes and Fothergill [1992] reports that in two- thirds of patients investigated for postmenopausal bleeding, no tissue was obtained.

Sensitivity of pipelle biopsy has been reported at between 70 and 84% [Antoni 1997, Gupta 1996] although Gimpleson and Goldchmit [1995] have reported that pipelle failed to make a correct diagnosis in 9.7-33% of cases and Archer [1991] that insufficient tissue for diagnosis was obtained in 24.5% of peri and postmenopausal women studied.

One possible reason for the low success rate of pipelle in the pilot group was because the majority of women were postmenopausal but when the figures are split into pre and postmenopausal, the failure rate is comparable as is the percentage where tissue was obtained. It is interesting that in the majority of women where pipelle was not possible, hysteroscopy could be performed. There are a number of potential explanations for this. Hysteroscopy was performed with women in lithotomy position which is the most ideal position for performing any gynaecological examination, although in the clinic setting this is not usually possible and in the pilot study women were not placed in this position for pipelle biopsy. Also, because there were no facilities to sterilise equipment for the pipelle biopsy (it was performed immediately following the ultrasound scan), a disposable speculum was used with no vulsellum. A vulsellum was available for use during the

hysteroscopy and is used to grasp the anterior lip of the cervix allowing the cervical canal to be straightened making it easier to enter the endometrial cavity. Presumably the use of a vulsellum at hysteroscopy accounted for some of the greater success with hysteroscopy. As sterilising was possible at the time of hysteroscopy, Cusco speculums were also used. Disposable speculums were of a standard size and one possible reason for the poor success rate was that if a smaller Cusco speculum could have been used, particularly in postmenopausal women who are more likely to have a degree of vaginal stenosis, then perhaps pipelle would have been easier. This explanation is not supported by the poor success rate in the premenopausal group as the majority of these women should be able to tolerate the bigger disposable speculum.

The ideal situation therefore would have been to perform the pipelle at the same visit as the hysteroscopy to standardise equipment and position but this was not ideal because if pipelle is performed first then it is likely to cause some uterine bleeding thus obscuring the view at hysteroscopy. When hysteroscopy is performed first the uterine cavity is filled with CO_2 gas. As pipelle works by suction, performing hysteroscopy first would make it difficult to obtain a sample using the pipelle as the suction device would be hampered by the presence of CO_2 in the cavity.

Whatever the possible explanations for the poor pipelle success rate, it remains that 18 women with thickened endometrium on ultrasound scan had no tissue obtained at pipelle. A negative pipelle is insufficient to exclude endometrial disease and these women would therefore have required further invasive investigation either by hysteroscopy or D & C. What is also concerning is that pipelle has been reported to sample on average only 4.2% (range 0-12.3%) of the endometrial surface even after the cannula is reintroduced three times [Habiba 1994, Rodrigues 1993]. If pipelle is likely to miss focal lesions because it samples only a small area of the cavity or associated polyps, then this questions the whole rationale behind its use in a group of women who are being screened primarily to exclude focal endometrial cancer. Pipelle is also no better tolerated than out-patient hysteroscopy which reinforces the conclusion that pipelle is not a good screening tool.

From these results it was concluded that pipelle biopsy should be abandoned in subsequent assessment of these women. There is no single technique which is 'all encompassing and

100% accurate' [al-Azzawi 1996] but transvaginal ultrasound appeared to be a good initial diagnostic method, so the remaining women recruited into the study had TV USS performed and only went on to out-patient hysteroscopy as a second line investigation if the scan was abnormal. Although it was anticipated from the results of the pilot study this would subject a number of women to out-patient hysteroscopy, a normal scan was reassuring and could be quickly, easily and relatively painlessly performed.

The low incidence of significant findings was surprising in view of other studies which had led us to expect a much higher incidence of abnormalities, including hyperplasia (simple and atypical) [Gal 1991, Uziely 1993, Lahti 1993, Cohen 1993(a), Cohen 1994(c)]. Some studies suggest up to 30% of women having abnormal endometrium. What is not clear from those studies however is what they classed as abnormal. This pilot study found no significant pathological findings. If, however, all the benign changes are classed as abnormal, then 31/120 = 25% of women fell into this category. Larger numbers of women would allow a more accurate comparison with other studies.

ENDOMETRIAL SCREENING

Introduction

Having established a screening protocol where all women had a transvaginal ultrasound scan performed and only went on to out-patient hysteroscopy if the scan was abnormal, the aim was to recruit more women into the study and obtain a control population. Because it is well accepted that women who have breast cancer are at a slightly higher risk of endometrial cancer than the general population, controls were women who had had breast cancer but had never been on tamoxifen.

The aim was to explore the relationship between tamoxifen and endometrial thickness on ultrasound scan in greater numbers of women to validate the results of the pilot study, and to compare endometrial thickness in women on tamoxifen with a series of control women. The true incidence of abnormal findings also needed to be further assessed as the pilot study suggested a low frequency of significant pathology, which is contrary to what has been reported in other studies. Larger numbers would confirm whether the incidence of significant pathology was low by chance because of small numbers in the pilot study or whether the low incidence was a true finding.

The results of this extended investigation would influence whether a screening programme was necessary.

Patients and Methods

A further 237 women and 130 controls were recruited into the study. The initial aim was to recruit a total of 500 women on tamoxifen and from patient enthusiasm this would have been possible, but time constraints made this impossible.

As a result of the pilot study these women all had transvaginal ultrasound scanning performed and only went on to out-patient hysteroscopy if the scan was abnormal. Pipelle biopsy was not performed.

Results

A total of 357 women on tamoxifen were studied. This included 120 women in the pilot study and 237 women in the extended study. Table 1 gives patient details and results for the remaining 237 women on tamoxifen (***details of the first 120 women can be found in Pilot Study: Results)*** and Table 2 gives details of the control population.

The following results pertain to all 357 study women and 130 controls.

In the study group, 42 were premenopausal and 315 postmenopausal. Endometrial thickness ranged from 1mm to 38mm, with a mean of 7.3mm and a median of 5mm, (95% C.I. 0.63). 212 women had an endometrium measuring <5mm or within accepted cycle limits in premenopausal women and 145 had thickened endometrium on scan.

Figures 1 and 2 show a normal uterus and endometrial thickness on tamoxifen and Figures 3, 4 and 5 show thickened endometrium on tamoxifen.

The control group of 130 women had endometrial thickness ranging from 1-10mm with a mean of 2.5mm and median of 2mm, (95% C.I. 0.34). 23 women were premenopausal and 107 postmenopausal. None of the postmenopausal control patients had endometrium >5mm in diameter or endometrium greater than accepted cycle limits in premenopausal women requiring hysteroscopy.

The endometrium of women on tamoxifen was significantly thicker than that of controls, 7.3mm compared with 2.5mm, $p<0.0001$, and is demonstrated graphically in Figure 6.

In control patients, length of time since diagnosis was used as a comparitor for time on tamoxifen. In this respect the groups are not statistically similar because women who were not taking tamoxifen were usually those whose diagnosis and treatment was many years ago before tamoxifen was widely used.

As with the pilot study there was a highly significant positive correlation between the length of time on tamoxifen and endometrial thickness, $p<0.0001$, demonstrated graphically in Figures 7 and 8 and the cumulative dose of tamoxifen and endometrial thickness, $p<0.0001$ which is demonstrated in Figure 9.

A summary of correlation between time on tamoxifen and endometrial thickness for the whole group is shown in Table 3. The group was split into 4 based on time on tamoxifen (0-2 years, 2-5 years, 5-10 years and 10+years) as these cut offs were deemed the most clinically relevant.

Table 3

Summary of correlation between time on tamoxifen and endometrial thickness.

Tamoxifen	No of Women	Mean endometrial thickness (mm) (Standard Error)	No thickened endometrium (%)
Whole group	357	7.3 (0.32)	145 (41%)
0-2 yrs	44	4.5 (0.45)	10 (23%)
2-5 yrs	118	5.8 (0.45)	32 (27%)
5-10 yrs	158	8.4 (0.53)	79 (50%)
10+yrs	37	10.8 (1.18)	24 (65%)
Controls	130	2.5 (0.17)	0

Of the women with thickened endometrium on scan 134 went on to have out-patient hysteroscopy. There were 11 failed hysteroscopies. Reasons for failure included cervical stenosis in 8 women, a cervical polyp in 2 women and a previous pelvic floor repair in 1 woman such that opening the speculum to view the cervix was impossible. All 11 women went on to have D & C to investigate this further.

Of 134 successful hysteroscopies, 61 women had normal or atrophic endometrium at hysteroscopy, giving a 46% false positive scan rate. This is a higher false positive rate than was found in the pilot study (38%).

The remaining 73 women, all had benign changes to account for thickened endometrium on TV USS.

These benign changes included:

Changes	No of Women
Polyps	21
Submucuous fibroids	6
Cystic endometrium	23
Oedematous endometrium	23

Numerous attempts were made to sample oedematous endometrium in the out-patient setting using pipelle, Sharman curette and other biopsy instruments; all were unsuccessful. The first 10 women with oedematous endometrium proceeded to D & C; no tissue was obtained in 7 and 3 had cystically dilated glands only. Subsequently this appearance was not investigated further.

No cancers were detected in the study group of 357 asymptomatic women and there were no women with thickened endometrium in the control group During the study period, 1 simple hyperplasia and 1 carcinoma was found in 2 women taking tamoxifen as adjuvant treatment for breast cancer who were referred for hysteroscopy as part of investigation of irregular vaginal bleeding.

An incidental finding was 20 (5.6%) ovarian cysts in the study group compared with 3 (2.3%) in the control group. This difference was not statistically significant (p=0.1528). All but one of the cysts detected were simple cysts (Figure 10), the other in the study group was multiloculated and suspicious of malignancy (Figure 11). This was removed and was a simple serous cystadenoma. All other women with ovarian cysts had repeat ultrasound scans at 3 monthly intervals and all cysts subsequently resolved spontaneously.

Table 1

Patient details and investigation results for the remaining 237 women on tamoxifen.

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
121.EA	55	post	5		60	36660
122.IA	75	post	5		106	64660
123.EA	49	post	1		27	16470
124.DA	73	post	5		34	20740
125.SA	37	pre	3		37	22570
126.FA	74	post	5		48	29280
127.CA	37	post	3		26	15860
128.GA	59	post	5		51	31110
129.SB	63	post	10	cystic	38	23180
130.BB	61	post	8	cystic	183	111630
131.MB	65	post	15	N/atrophic	135	82350
132.AB	63	post	7	N/atrophic	157	95770
133.PB	57	post	4		134	81740
134.EB	59	post	12	N/atrophic	51	31110
135.EB	75	post	1		25	15250
136.EB	44	pre	4		38	23180
137.KB	46	pre	4		75	45750
138.AB	63	post	5		60	36600
139.MB	69	post	9	N/atrophic	89	54290
140.AB	50	post	1		37	22570
141.DB	62	post	3		27	16470
142.MB	49	pre	5		99	60390
143.EB	74	post	3		97	59170
144.SB	56	post	4		120	73200

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
145.HB	70	post	14	oedematous	50	30500
146.CB	67	post	18	oedematous	61	37210
147.MB	48	pre	1		50	30500
148.AC	72	post	20	cystic	122	74420
149.EC	66	post	3		75	45750
150.SC	59	post	4		117	71370
151.JC	54	pre	1		38	23180
152.SC	60	post	4		86	52460
153.IC	72	post	14	cystic	74	45140
154.PC	57	post	2		27	16470
155.LC	58	post	4		74	45140
156.BC	53	post	3		14	8540
157.HC	60	post	5		73	44530
158.JC	73	post	7	cystic	75	45750
159.IC	52	post	4		23	14030
160.LC	71	post	2		73	44530
161.NC	68	post	4		71	43310
162.IC	75	post	4		57	34770
163.MC	63	post	18	oedematous	75	45750
164.AC	75	post	5		64	39040
165.EC	52	post	4		55	33550
166.MD	76	post	26	N/atrophic	113	68930
167.VD	57	post	4		62	37820
168.PD	41	pre	4		50	30500
169.MD	66	post	7	unable to do Cx stenosis	58	35380
170.ID	55	post	4		13	7930

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
171.CD	62	post	13	N/atrophic	83	50630
172.ED	61	post	14	N/atrophic	18	10980
173.GD	61	post	5		37	22570
174.CD	67	post	5		91	55510
175.MD	59	post	4		182	111020
176.PD	65	post	3		138	84180
177.RE	71	post	14	oedematous	109	66490
178.EE	52	post	7	N/atrophic	62	37820
179.FF	40	post	3		27	16470
180.IF	62	post	1		25	15250
181.JF	53	post	9	submucous fibroid	93	56730
182.MF	41	post	4		25	15250
183.EF	65	post	5		28	17080
184.MF	67	post	11	N/atrophic	26	15860
185.SF	56	post	3		52	31720
186.TF	52	post	5		48	29280
187.SF	75	post	2		62	37820
188.MF	51	post	3		98	59780
189.MF	73	post	4		75	45750
190.JF	44	post	3		51	31110
191.HF	70	post	4		98	59780
192.NG	50	post	4		22	13420
193.MG	59	post	7	N/atrophic	48	29280
194.DG	66	post	15	polyp	64	39040
195.SG	75	post	4		61	37210
196.JG	62	post	5		26	15860

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
197.EG	60	post	3		74	45140
198.SG	39	pre	3		24	14640
199.EH	66	post	36	polyp	61	37210
200.MH	66	post	14	cystic	115	70150
201.PH	48	pre	14	polyp	49	29890
202.MH	73	post	10	N/atrophic	113	68930
203.AH	57	post	5		65	39650
204.CH	37	pre	3		48	29280
205.MH	54	pre	30	cystic	44	26840
206.MH	48	pre	4		35	21350
207.EH	62	post	5		74	45140
208.CH	70	post	3		36	21960
209.CH	68	post	4		63	38430
210.DH	54	post	2		12	7300
211.AI	46	post	4		28	17080
212.SI	59	post	15	N/atrophic	12	7300
213.JJ	43	pre	3		18	10980
214.MJ	61	post	3		60	36600
215.MJ	56	post	4		63	38430
216.SJ	51	post	17	N/atrophic	91	55510
217.MJ	65	post	4		52	31720
218.NJ	68	post	3		68	41480
219.AJ	68	post	2		14	8540
220.MJ	64	post	3		49	29890
221.MK	48	pre	5		84	51240
222.JK	62	post	3		26	15860
223.PK	54	post	5		61	37210

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
224.AK	63	post	4		33	20130
225.GK	52	post	3		126	76860
226.TK	63	post	2		75	45750
227.WL	51	pre	5		136	82960
228.EL	50	post	4		62	37820
229.AL	59	post	5		97	59170
230.DL	72	post	5		22	13420
231.ML	64	post	8	N/atrophic	35	21350
232.PL	51	post	11	N/atrophic	49	29890
233.AL	50	post	6	N/atrophic	61	37210
234.GL	51	post	4		72	43920
235.JL	67	post	2		77	46970
236.RM	53	post	14	cystic	134	81740
237.MM	73	post	7	N/atrophic	51	31110
238.EM	70	post	12	N/atrophic	122	74420
239.SM	67	post	4		71	43310
240.MM	56	post	5		85	51850
241.EM	65	post	5		28	17080
242.IM	73	post	31	unable to do Cx stenosis	85	51850
243.AM	58	post	10	cystic	74	45140
244.CM	71	post	5		97	59170
245.IM	68	post	9	polyp	80	48800
246.GM	36	pre	3		16	9760
247.AM	66	post	3		66	40260
248.NM	64	post	5		26	15860
249.AM	74	post	15	N/atrophic	85	51850

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
250.IM	51	pre	2		62	37820
251.SM	43	post	3		26	15860
252.JM	62	post	26	N/atrophic	122	74420
253.JM	57	post	4		98	59780
254.SM	69	post	5		61	37210
255.JM	67	post	5		82	50020
256.JM	57	post	7	N/atrophic	72	43920
257.MM	47	pre	8	submucous fibroid	73	44530
258.IM	61	post	10	oedematous	131	79910
259.HM	57	post	3		15	9150
260.OM	48	pre	12	N/atrophic	62	37820
261.MM	57	post	5		20	12200
262.AM	61	post	5		50	30500
263.BN	48	post	12	oedematous	31	18910
264.MN	59	post	4		63	38430
265.EN	60	post	5		25	15250
266.AN	60	post	2		37	22570
267.FO	65	post	5		64	39040
268.EO	53	post	4		51	31110
269.JP	44	pre	10	oedematous	46	28060
270.CP	59	post	4		60	36600
271.KP	48	post	5		61	37210
272.EP	68	post	16	oedematous	54	32940
273.MP	59	post	4		61	37210
274.MP	62	post	3		12	7300
275.JP	74	post	5		111	67710

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
276.MR	63	post	11	oedematous	86	52460
277.GR	73	post	19	oedematous	134	81740
278.LR	59	post	2		53	32330
279.JR	64	post	9	N/atrophic	32	19520
280.AR	42	post	5		153	93330
281.MR	61	post	11	N/atrophic	109	66490
282.JR	65	post	6	unable to do Cx stenosis	104	63440
283.IR	58	post	18	polyp	124	75640
284.MR	74	post	4		9	5490
285.HR	52	post	5		17	10370
286.JR	61	post	4		37	22570
287.ER	60	post	2		32	19520
288.CR	57	post	2		33	20130
289.RS	62	post	3		41	25010
290.MS	42	pre	8	N/atrophic	100	61000
291.AS	47	post	2		27	16470
292.TS	51	pre	2		62	37820
293.ES	50	pre	4		39	23790
294.SS	73	post	19	cystic	49	29890
295.TS	60	post	2		21	12810
296.KS	38	pre	5		61	37210
297.MS	63	post	8	N/atrophic	26	15860
298.RS	69	post	15	N/atrophic	86	52460
299.GS	61	post	8	oedematous	38	23180
300.CS	76	post	5		128	78080
301.MS	51	pre	5		64	39040

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
302.AS	45	post	4		52	31720
303.RS	49	post	11	N/atrophic	120	73200
304.AS	68	post	5		37	22570
305.JS	69	post	4		128	78080
306.KS	45	pre	5		32	19520
307.CS	54	post	9	cystic	69	42090
308.SS	70	post	7	cystic	23	14030
309.RS	52	post	2		18	10980
310.MS	69	post	3		120	73000
311.AS	47	pre	5		38	23180
312.AS	61	post	4		182	111020
313.IS	69	post	13	N/atrophic	157	95770
314.ES	75	post	11	cystic	20	12200
315.NS	54	post	3		38	23180
316.CS	54	post	3		136	82960
317.AT	49	post	7	N/atrophic	110	67100
318.MT	52	post	4		73	44530
319.RT	70	post	13	N/atrophic	60	36600
320.CT	75	post	4		121	73810
321.CT	54	post	2		13	7930
322.HW	62	post	3		26	15860
323.MW	67	post	17	N/atrophic	111	67710
324.LW	64	post	4		86	52460
325.MW	53	post	5		52	31720
326.YW	53	post	4		12	7300
327.WW	67	post	2		25	15250
328.JW	49	post	3		33	20130

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
329.JW	63	post	5		28	17080
330.VW	63	post	2		83	50630
331.GY	68	post	4		71	43310
332.AD	71	post	9	unable to do Cx polyp	160	97600
333.MG	74	post	8	declined hysteroscopy	61	37210
334.CS	68	post	7	unable to do Cx stenosis	73	44530
335.AS	76	post	2		96	58560
336.IW	85	post	6	unable to do Cx stenosis	70	42700
337.JT	50	pre	12	oedematous	54	32940
338.EH	68	post	15	N/atrophic	99	60390
339.MR	53	post	16	N/atrophic	148	90280
340.EC	74	post	11	cystic	121	73810
341.MD	59	post	7	cystic	67	40870
342.AM	66	post	6	N/atrophic	108	65880
343.MM	68	post	7	N/atrophic	109	66490
344.CM	62	post	19	N/atrophic	98	59780
345.MD	70	post	16	N/atrophic	99	60390
346.YH	57	post	9	N/atrophic	61	37210
347.HS	61	post	8	N/atrophic	76	46360
348.JP	68	post	10	N/atrophic	62	37820
349.ED	65	post	23	N/atrophic	74	45140
350.JF	67	post	15	oedematous	65	39650
351.IW	71	post	11	N/atrophic	81	49410
352.JH	65	post	23	N/atrophic	191	116510

Patient	Age	Menopausal State	TV USS (mm)	Hysteroscopy Findings	Time on Tamoxifen	Cumulative Dose Tamoxifen mg
353.NT	65	post	11	oedematous	109	66490
354.ES	64	post	8	N/atrophic	63	38430
355.JK	74	post	12	cystic	62	37820
356.RB	71	post	7	N/atrophic	123	75030
357.MM	56	post	18	N/atrophic	144	87840

Table 2

Control patient details and results.

Patient	Age	Menopausal State	TV USS (mm)	Time Since Diagnosis (mths)
1. RCH	54	post	1	85
2. WP	52	post	2	112
3. IW	68	post	1	198
4. AH	59	post	1	127
5. FS	42	pre	3	113
6. SM	61	post	2	13
7. PM	67	post	2	162
8. AJ	59	post	1	198
9. MM	57	post	1	148
10. IW	57	post	1	114
11. MG	63	post	1	185
12. MM	62	post	2	125
13. JR	67	post	2	241
14. ES	55	post	2	185
15. ME	70	post	3	159
16. SJ	45	pre	2	137
17. IG	59	post	1	199
18. CL	59	post	3	14
19. EM	54	post	2	117
20. EH	48	post	2	108
21. RB	50	post	1	80
22. CH	37	pre	2	77
23. CR	73	post	2	195
24. KT	57	post	2	24

Patient	Age	Menopausal State	TV USS (mm)	Time Since Diagnosis (mths)
25. EM	47	pre	6	160
26. AB	69	post	2	44
27. MA	61	post	1	195
28. EA	61	post	2	195
29. MM	31	pre	7	14
30. AN	69	post	1	197
31. BA	57	post	4	62
32. RW	75	post	1	176
33. ER	68	post	2	213
34. MM	49	pre	7	178
35. BL	64	post	2	138
36. ER	54	post	1	272
37. JW	60	post	1	96
38. MM	67	post	1	146
39. AC	70	post	1	72
40. BM	61	post	1	70
41. CP	66	post	1	37
42. MC	52	post	2	9
43. MM	42	pre	9	25
44. MD	55	post	3	62
45. MB	49	post	2	95
46. EM	60	post	3	136
47. IR	60	post	3	133
48. LG	41	pre	3	101
49. RK	38	pre	3	25
50. AB	57	post	1	179
51. DB	55	post	1	106
52. AG	57	post	1	80

Patient	Age	Menopausal State	TV USS (mm)	Time Since Diagnosis (mths)
53. MB	65	post	1	263
54. AB	51	post	2	122
55. LA	43	pre	3	25
56. IC	47	post	1	76
57. AB	58	post	3	169
58. MP	53	post	1	90
59. MC	53	post	1	85
60. JS	44	pre	6	112
61. ES	65	post	1	87
62. HM	54	post	2	34
63. MP	68	post	2	90
64. MM	58	post	4	123
65. SP	53	pre	7	24
66. MB	59	post	2	78
67. MM	57	post	1	237
68. MA	67	post	1	248
69. SB	70	post	3	286
70. CM	66	post	4	27
71. AH	41	pre	2	73
72. IW	75	post	4	176
73. MW	57	post	2	114
74. IG	59	post	5	210
75. AW	69	post	1	169
76. AS	50	post	3	71
77. JM	53	post	1	114
78. JD	66	post	2	25
79. BW	45	pre	5	157
80. AS	63	post	2	154

Patient	Age	Menopausal State	TV USS (mm)	Time Since Diagnosis (mths)
81. MA	62	post	1	72
82. EG	74	post	3	164
83. SM	62	post	1	173
84. MM	66	post	3	116
85. MM	62	post	2	35
86. MF	71	post	2	81
87. MA	58	post	1	62
88. AH	66	post	2	168
89. MG	72	post	2	38
90. PT	67	post	2	201
91. DM	41	pre	6	93
92. AS	57	post	2	70
93. AS	53	post	1	99
94. JC	54	post	1	28
95. IV	47	post	1	106
96. Kl	63	post	3	47
97. DR	57	post	2	71
98. MG	54	post	3	143
99. MP	63	post	1	30
100. PM	47	pre	4	90
101. MM	48	post	2	97
102. EM	50	post	2	74
103. NL	59	post	2	150
104. PL	69	post	1	133
105. EB	52	post	3	27
106. BM	48	post	2	82
107. MB	66	post	2	111
108. MM	59	post	1	69

Patient	Age	Menopausal State	TV USS (mm)	Time Since Diagnosis (mths)
109. BW	52	pre	3	98
110. AM	41	pre	3	102
111. ES	60	post	2	58
112. JR	46	pre	9	187
113. CR	61	post	1	113
114. IO	52	post	3	14
115. EM	56	post	2	86
116. PC	50	post	1	221
117. HA	61	post	1	52
118. MW	68	post	3	192
119. WB	59	post	3	172
120. SM	51	post	2	219
121. MS	66	post	2	114
122. MR	48	pre	4	102
123. EC	55	post	1	106
124. MD	54	post	2	119
125. DW	51	post	3	94
126. JL	46	post	1	58
127. SN	54	post	2	162
128. MS	44	pre	5	75
129. NM	42	pre	8	99
130. JK	57	post	1	113

2 examples of a normal uterus and endometrial thickness on TV USS.

Figure 1

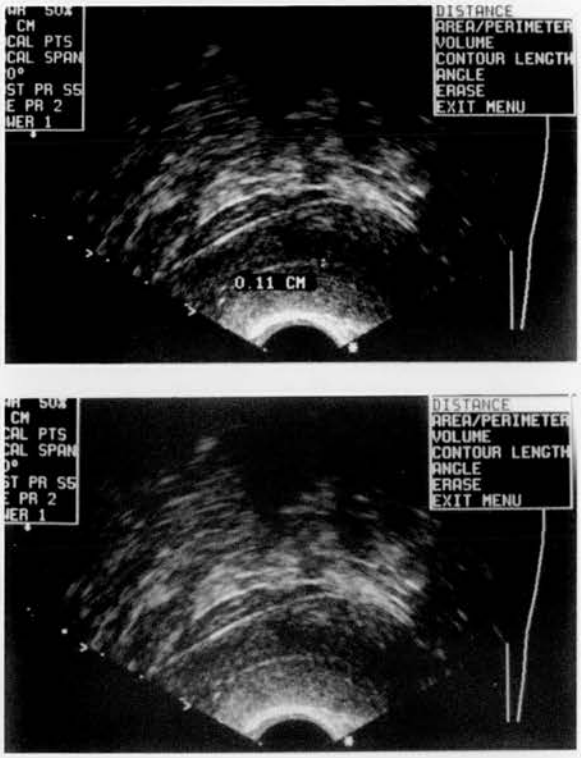
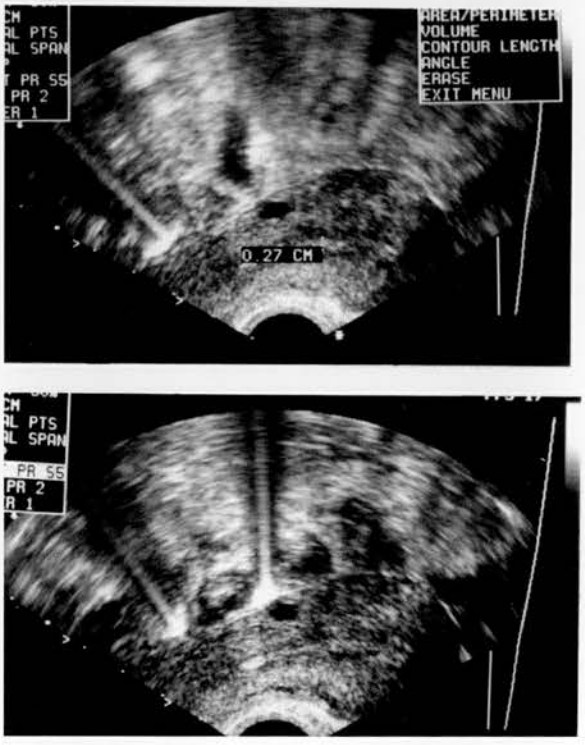


Figure 2



2 examples of thickened cystic endometrium on TV USS.

Figure 3

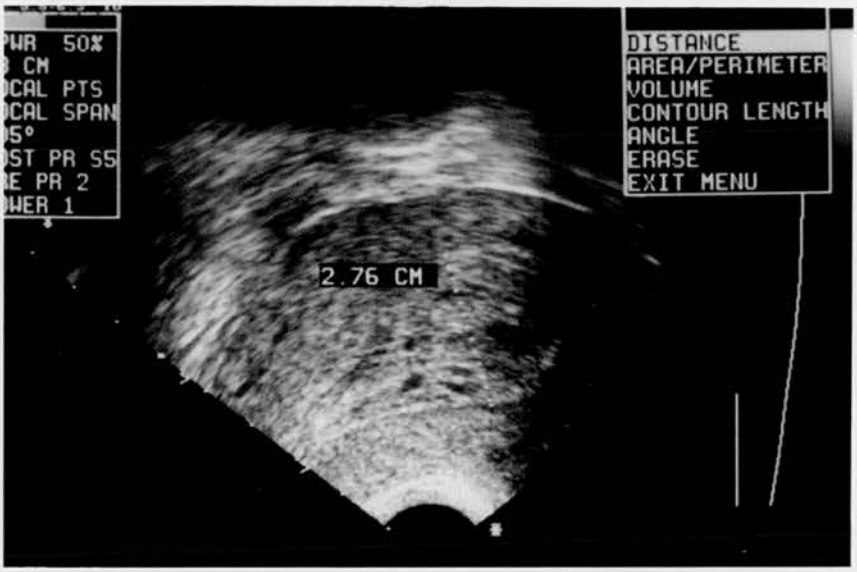


Figure 4

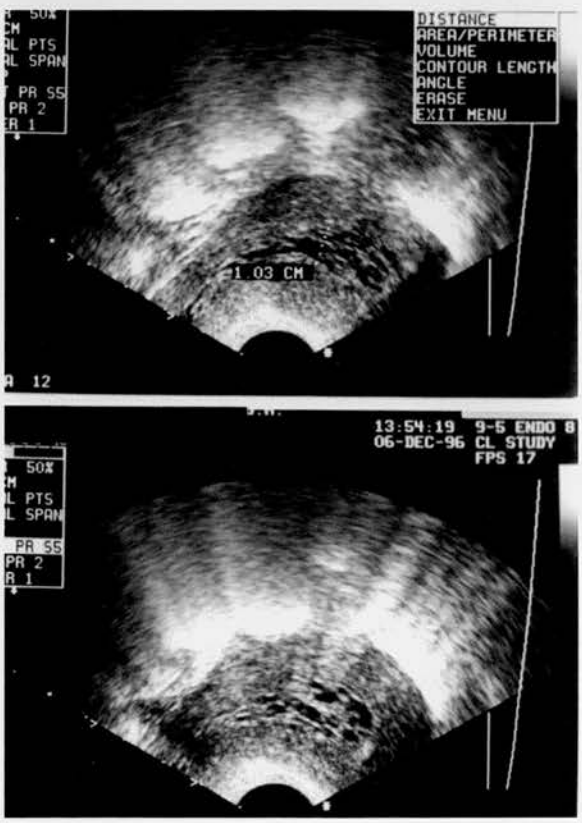


Figure 5

Thickened cystic endometrium on TV USS.

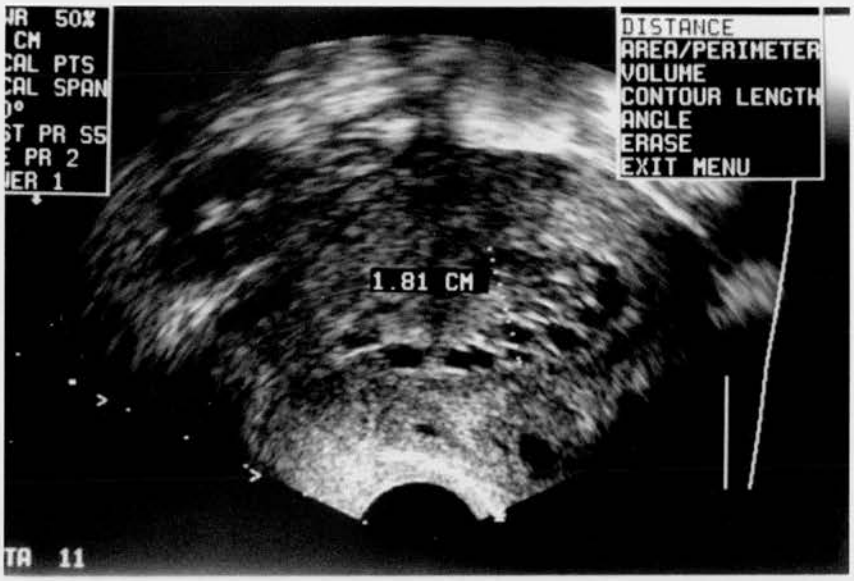


Figure 6
Mean endometrial thickness of study versus control group.

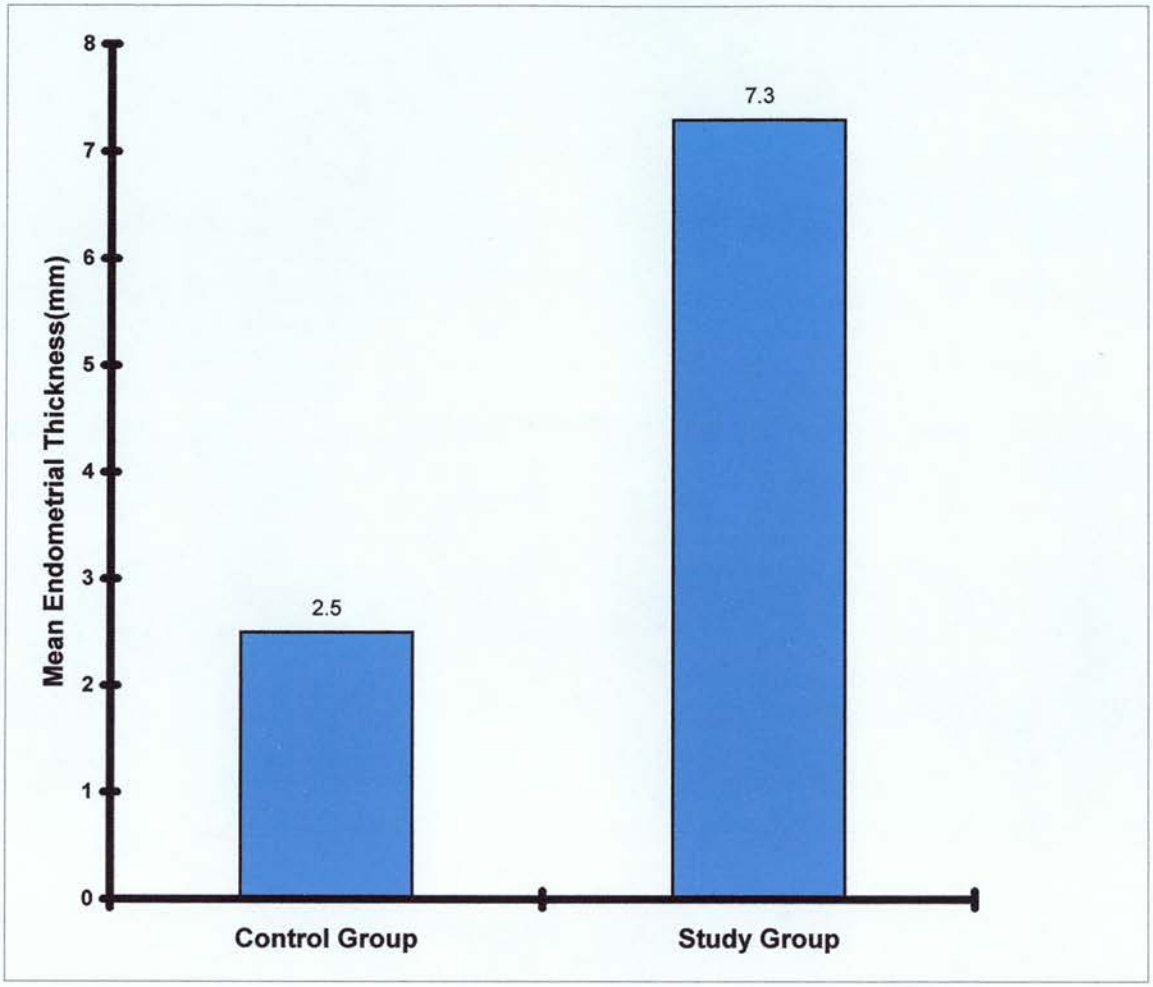
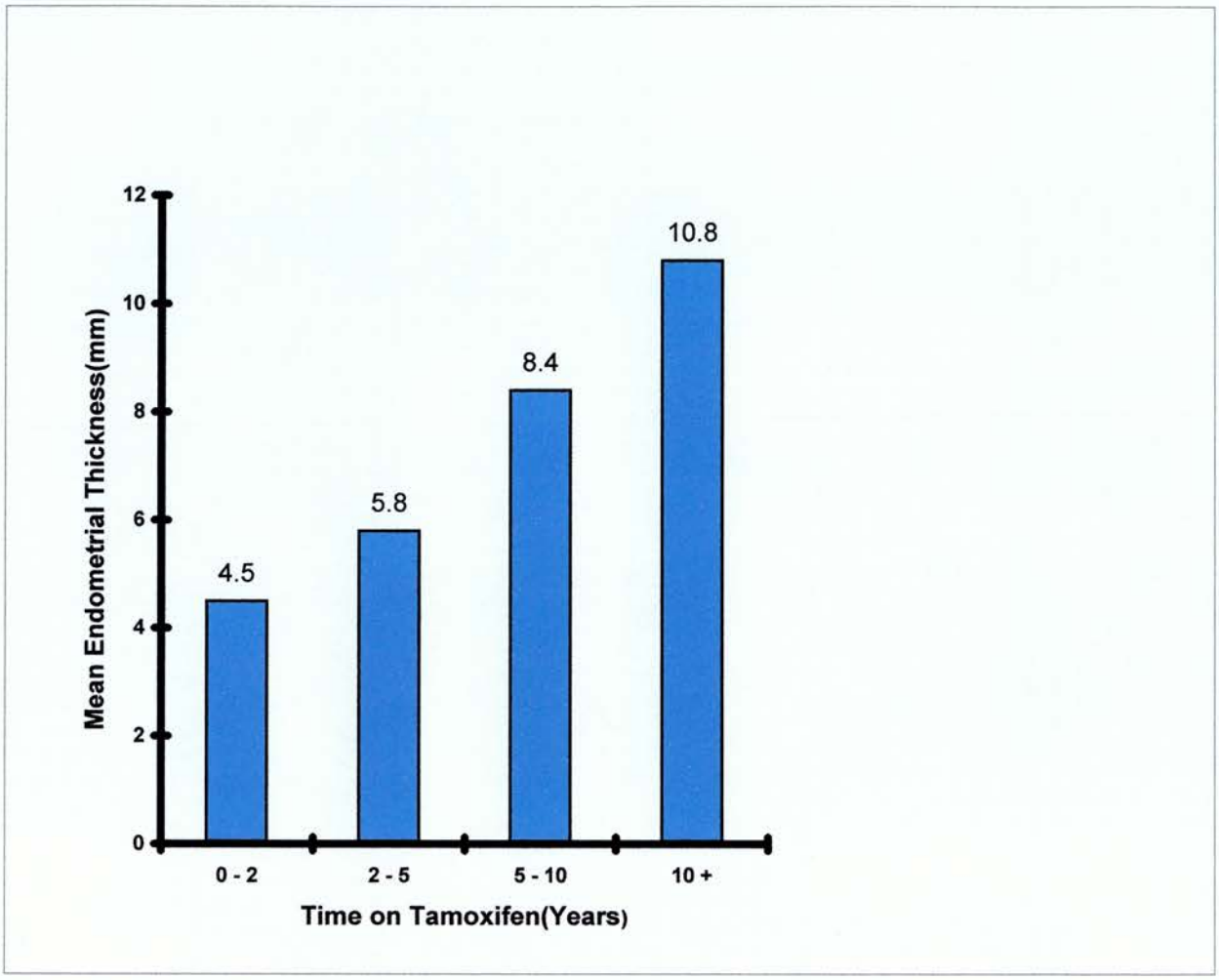


Figure 7
Relationship between mean endometrial thickness and time on tamoxifen.



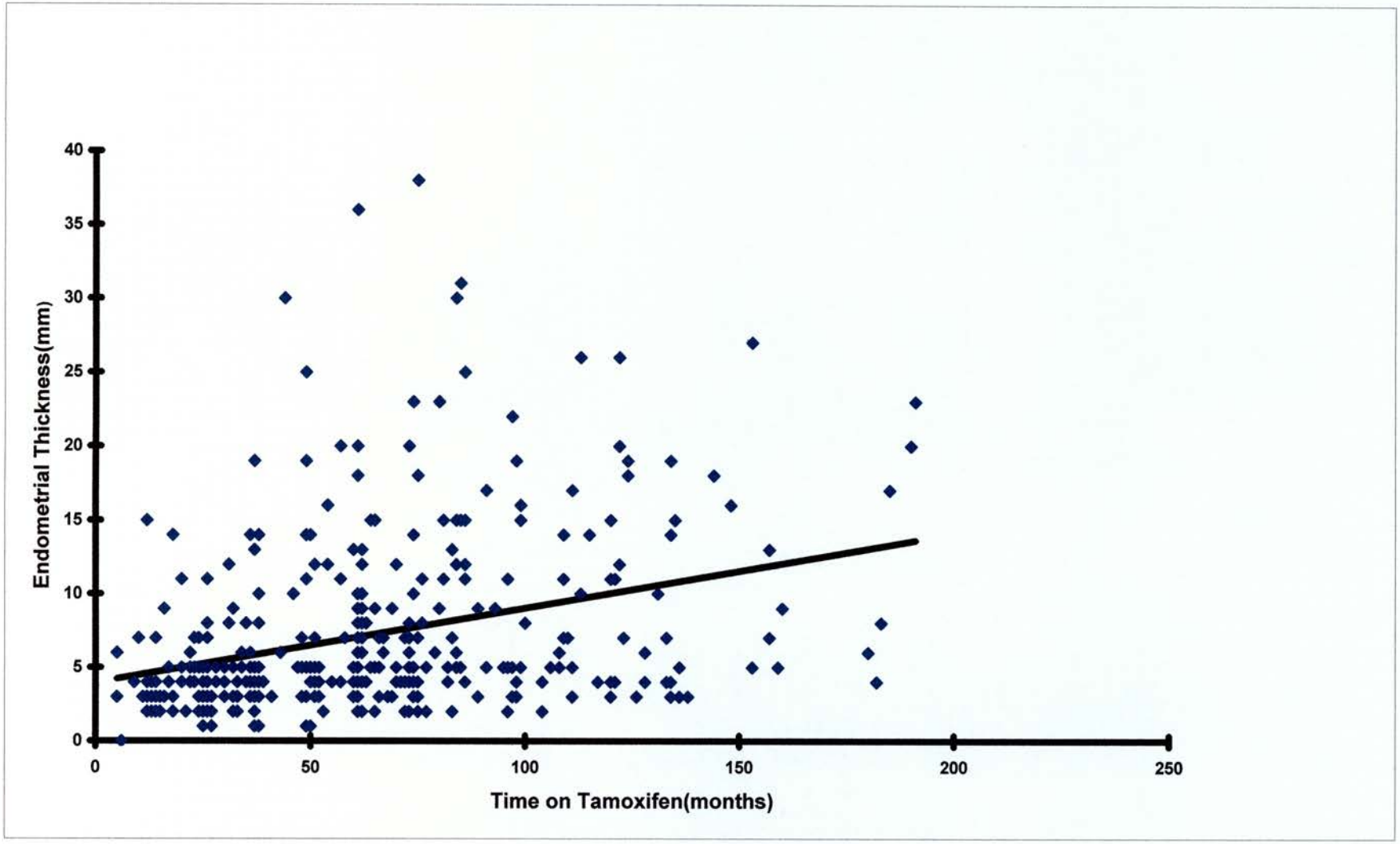


Figure 8
Relationship between endometrial thickness and time on tamoxifen.

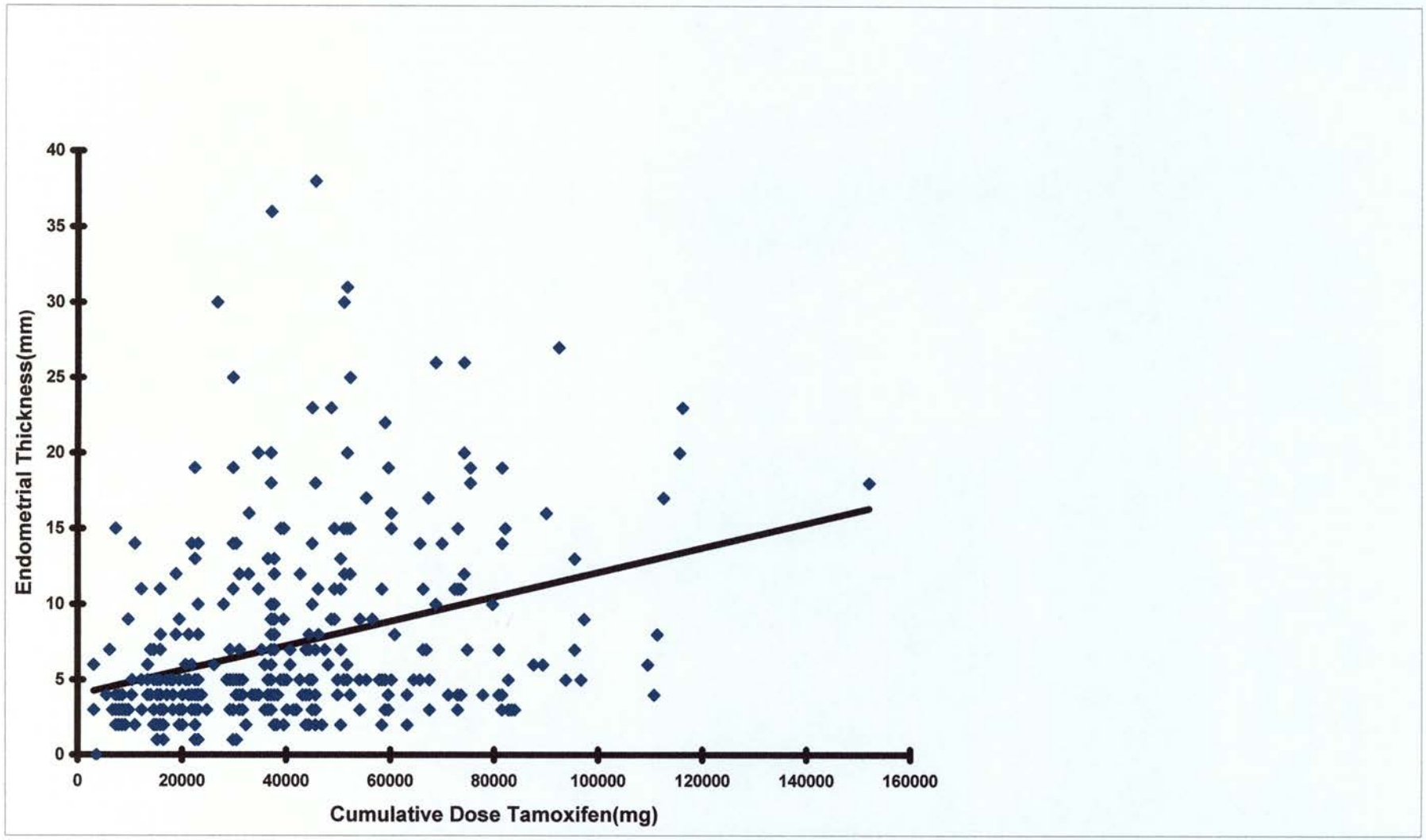


Figure 9
Relationship between endometrial thickness and cumulative dose of tamoxifen.

Figure 10

Simple ovarian cyst on tamoxifen.

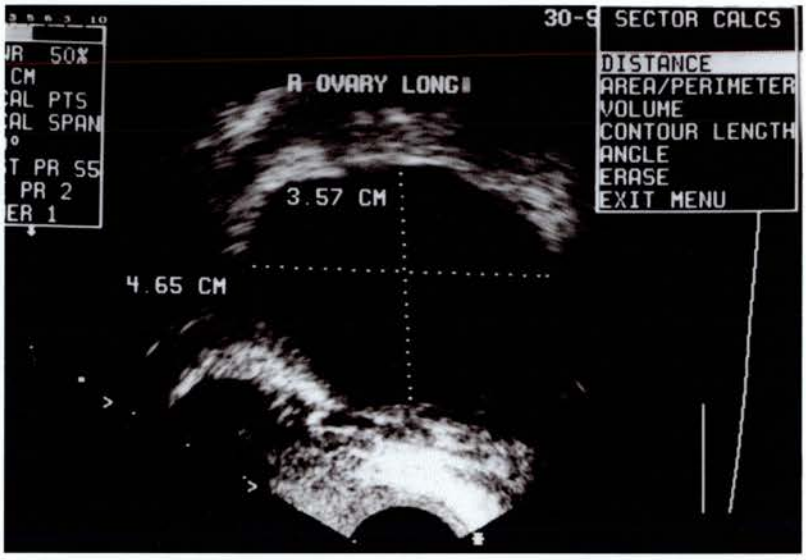
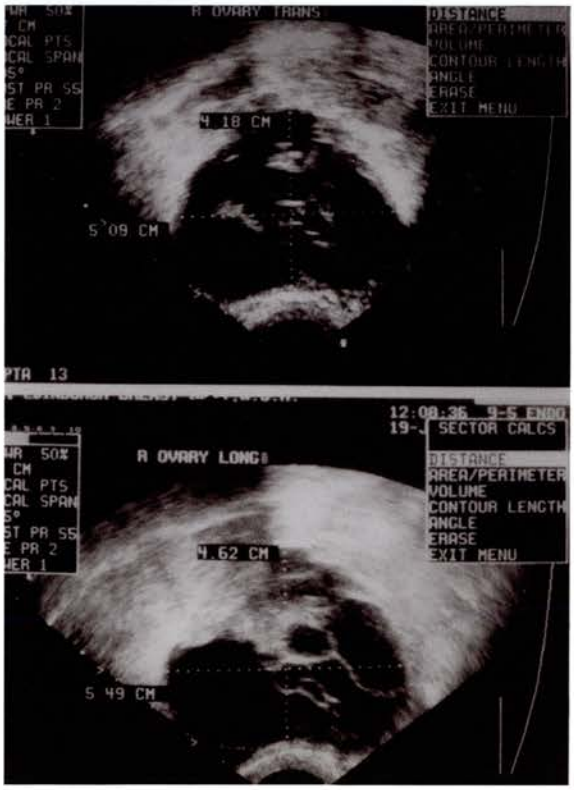


Figure 11

Multiloculated ovarian cyst on tamoxifen.



Discussion

The main aim of this part of the research project was to assess the incidence of endometrial abnormalities in asymptomatic women on tamoxifen and address whether screening was valuable and, if so, what the optimal screening protocol was. There is clear evidence of an increased risk of endomentrial cancer in women taking tamoxifen (from 1:1000 women/year to 2:1000 women/year) [Assikis 1996, Fisher 1994, Fornander 1989, Cohen 1995(a), Cohen 1994(a), Friedl 1994, Jaiyesimi 1995, Rutqvist 1987, Sismondi1994] but it is accepted that the benefits from tamoxifen in reducing recurrence both locally and metastatic and reducing the number of contralateral breast cancers is greater than the number of extra women who develop endometrial cancer. Recent studies have also demonstrated a reduction in breast cancer incidence with prophylactic use of tamoxifen and it has a produce licence in the USA for prevention of breast cancer in high risk women. As the risks of endometrial cancer do not warrant cessation of the drug, the role of endometrial screening needs to be assessed.

Up to 30% of women taking tamoxifen have been reported to develop endometrial abnormalities ranging from benign polyps and simple hyperplasia to atypical hyperplasia and cancer [Cohen 1993, Gal 1991, Sismondi 1994, Cohen 1993, De Muylder 1991, Neven 1991, Rullo 1993, Fotiou 1998, Cohen 1999]. This is an alarmingly high incidence of abnormalities and was one of the main reasons for undertaking the present study. However, from published reports it is unclear whether the abnormalities which develop on tamoxifen are related to cumulative dose and are therefore time dependent, or whether the abnormalities have been identified in mainly symptomatic women as opposed to the present study which investigates asymptomatic women. Cohen [1999] has reported recently that endometrial pathology is more likely to be diagnosed in symptomatic women on tamoxifen.

If the current study had confirmed a similarly high incidence of significant abnormalities in asymptomatic women on tamoxifen then it may have proved necessary to implement a screening programme for all women on tamoxifen - whether they were taking tamoxifen as treatment for their breast cancer or as part of the ongoing tamoxifen prevention studies.

This would have significant economic implications and thus the results of the present study were important.

The pilot study detected an incidence of benign changes in 25% of women and this is in keeping with the high incidence of abnormalities reported in the literature but none of these abnormalities were significant, consisting only of polyps and submucous fibroids in 11% and cystic or oedematous endometrium in 14%. The significance of these latter findings is unclear and whether they can be regarded as truly 'abnormal' is doubtful.

This study aimed to confirm (or refute) the findings of the pilot study by enrolling larger numbers. The pilot study investigated the validity and acceptability to patients of transvaginal ultrasound scanning, out-patient hysteroscopy and pipelle biopsy and concluded that patients enrolled into the study thereafter would have transvaginal ultrasound scanning performed and only go on to out-patient hysteroscopy if the scan was abnormal. The study group of 357 women on tamoxifen included the 120 women from the pilot study. Because of common risk factors between breast cancer and endometrial cancer, a control population of 130 women, all of whom had breast cancer but had never taken tamoxifen, were chosen. Because it has been routine practice for the last 10 years to give all postmenopausal women tamoxifen, control patients tended to have been treated earlier in time than study patients giving 2 non comparable groups with reference to time since diagnosis.

In the 357 women with breast cancer on adjuvant tamoxifen, 145 (41%) had apparent endometrial thickening on transvaginal ultrasound scan and therefore as part of the protocol went on to have out-patient hysteroscopy performed. At hysteroscopy 61 of these women (46%) were found to have atrophic/normal endometrium, 21 had polyps, 6 submucous fibroids, 23 cystic endometrium and 23 oedematous endometrium. There were no cases of hyperplasia or cancer detected in the study group although 1 patient with simple hyperplasia and 1 patient with cancer was detected in 2 symptomatic women on tamoxifen referred for out-patient hysteroscopy. The risk of endometrial cancer in women on tamoxifen is of the order of 2:1000 women/year and therefore it could be argued that the study population of 357 women was not large enough to detect cases of endometrial cancer but from the literature other significant abnormalities, eg hyperplasia, would have been

expected in a population of this size. TV USS has a reported sensitivity of 54-97% and specificity of 48-90% [Alcazar 1996, Cacciatore 1994, Dijkhuizen 1996, Haller 1996, Towbin 1996, Langer 1997] and hysteroscopy a sensitivity of 79-100% and specificity of 93-100% [Alcazar 1996, Cacciatore 1994, Towbin 1996, Haller 1996]. In combination, it is highly unlikely that any significant lesions were missed to account for the lack of abnormalities detected in this study population. 212 women (59%) had normal endometrium on TV USS and therefore could be reassured and no further investigation was required. The endometrium of women on tamoxifen was significantly thicker on TV USS than that of controls (mean 7.3mm on tamoxifen versus 2.5mm control; $p<0.0001$) and this is a finding supported by others [Rayter 1994, Kedar 1994, Lahti 1993]. None of the control patients had thickened endometrium as measured by TV USS requiring further investigation.

61 of the 145 women on tamoxifen with thickened endometrium on TV USS had atrophic/normal endometrium at hysteroscopy. Although it had been established from the pilot study that TV USS was sensitive, it was not highly specific (66%) with a 38% false positive scan rate in the pilot study. TV USS was chosen as an initial screening tool because it was simple, cheap, quick to perform and highly acceptable to patients. However, the main study confirmed an overall 46% false positive scan rate thus subjecting large numbers of women to unnecessary further invasive investigation and accompanying anxiety without significant benefit. This is particularly important in a group of women who have already been through the trauma of a diagnosis and treatment of breast cancer. The high false positive rate questions the use of TV USS in a mass screening programme and indicates it would cause unnecessary anxiety to a large number of women without significant disease.

Benign abnormalities (polyps and submucuous fibroids) were detected in 27/145 women who had thickened endometrium on USS. The incidence of polyps in asymptomatic women in the general population is unknown so the significance of these asymptomatic polyps is unclear. In general gynaecological practise, symptomatic polyps would be removed - in premenopausal women because they cause dysfunctional uterine bleeding and in postmenopausal women, because of the concern they may have malignant change as a cause for postmenopausal bleeding. We know from postmortem studies that the incidence

of occult endometrial cancer is significant [Horwitz 1981] and it would be logical to assume therefore that not all polyps are symptomatic. Following discussion with the consultant gynaecologist involved in the present study, women with polyps were offered either D&C to remove the polyp or repeat hysteroscopy 6 months later to review the polyp. Recent studies have suggested that there is a higher incidence of malignant foci developing in polyps of women on tamoxifen than in the general population [Ismail 1994(a),(b), Ramondetta 1999, Nouvo 1989] therefore if patients decided not to have their asymptomatic polyp removed, repeat hysteroscopy was deemed necessary. 12 women opted for removal and all polyps were benign and 9 opted for repeat hysteroscopy. At repeat hysteroscopy all women remained asymptomatic and none of the polyps had changed in size or character. All hysteroscopies were recorded on videotape so initial and repeat hysteroscopy could be compared.

It was concluded that none of these benign abnormalities could be considered as significant abnormalities.

Cystic/oedematous endometrium was found in 46 women. Again, it is questionable whether this is significant or even whether this should be classed as abnormal in women on tamoxifen. Cystic appearances of the endometrium on TV USS have been previously reported in women taking tamoxifen [Cohen 1996, Anteby 1992, Aleem 1995, Ciatto 1994, Goldstein 1994, Hulka 1993]. At hysteroscopy most of the endometrium appeared benign and consisted of several thin-walled cysts filled with clear fluid which tended to rupture whilst attempting to biopsy them. The 'oedematous' appearance has not been previously described although some have described an unusual appearance at hysteroscopy with the absence of pathology from women on tamoxifen [Touraine 1995, Goldstein 1994]. These women had thickened endometrium at USS and at hysteroscopy the endometrium appeared thickened and 'boggy'. The appearance at hysteroscopy suggested that biopsy of the endometrium would be straightforward but despite trying various biopsy techniques and a variety of instruments, no tissue could be obtained. In view of this, the first 10 women with this appearance proceeded to D&C as there was concern both about the underlying pathology and the failure to obtain a tissue diagnosis. Surprisingly, even at D&C no tissue was obtained in 7 out of 10 of these women (indicating atrophic endometrium) and the histology of the remaining 3 patients showed cystically dilated glands but no other

endometrial abnormality. In view of these findings it was felt inappropriate to subject the other 13 asymptomatic women with the same hysteroscopic appearance to further investigation.

It has been suggested that a thickened cystic appearance on TV USS may be caused by endometrial serosal or superficial myometrial oedema. The appearance at hysteroscopy and lack of tissue obtained is consistent with oedema causing the superficial endometrium to appear thickened when in fact it is atrophic. It could be hypothesised that discrete cysts seen in some women is one end of a spectrum caused by tamoxifen, and perhaps the initial event, which subsequently develops into the more generalised oedematous appearance seen at hysteroscopy, which in the 3 women where tissue was obtained corresponds histologically to cystically dilated glands as the only 'abnormality'. However, in the patients studied, there was no difference in the mean length of time on tamoxifen between those with cystic or oedematous endometrium to support this hypothesis. There is as yet no sound scientific explanation for these appearances but they do appear to be specific to tamoxifen use.

Unfortunately, there were no specific features on TV USS which predicted whether the apparently thickened endometrium was atrophic/normal, oedematous, cystic or whether other benign changes eg polyps would be found at hysteroscopy. These results from TV USS show that it does not consistently identify true endometrial thickness in women on tamoxifen and casts further doubt about its role as a screening tool. Others have also concluded that USS from women on tamoxifen did not distinguish atrophy from other benign processes [McGonicle 1998, Mourits 1998].

The pilot study found a highly significant ($p<0.0001$) positive correlation between endometrial thickness as measured by TV USS and length of time on tamoxifen. This correlation was confirmed in the main study and the association remained highly significant ($p<0.0001$). However, apparently thickened endometrium on TV USS does not necessarily indicate either that the endometrium is actually thickened or that there is a higher incidence of benign lesions in asymptomatic women who take tamoxifen for long periods. Some have suggested that the risk of carcinoma is time and dose dependent [Anderson 1991, Fornander 1989, Nayfield 1991] but others have disagreed with this [Cohen 1994(b), Neven 1994(a), Plourde 1995] and the NSABP findings suggest that the incidence remains constant

over time on the drug. Caution is therefore required when interpreting TV USS in women on tamoxifen and when TV USS in such a patient suggests endometrial thickening it would seem appropriate to proceed to out-patient hysteroscopy as the next investigation rather than in-patient general anaesthetic procedures such as D&C to sample the endometrium as a large number of these women will have no significant endometrial pathology.

A direct correlation of apparent endometrial thickening with increasing duration of tamoxifen was a subject which required further study. As sampling the endometrium was a problem, it was considered the only way to investigate this further was to study the endometrium of women who had undergone hysterectomy whilst on tamoxifen for the treatment of breast cancer. This work is described in detail in a subsequent chapter (***Endometrial Pathology***).

Although there was a highly significant correlation between length of time on tamoxifen and endometrial thickness on TVUSS, there remained wide individual variation between women for which no explanation could be found, eg patient 144 was on tamoxifen for 120 months and she had an endometrium measuring 4mm while patient 148 who had been taking tamoxifen for 122 months and had an endometrium measuring 22mm. Nonetheless, the longer a woman is on tamoxifen, the more likely she is to have apparent endometrial thickening on TV USS and to require further investigation if regular screening were to be introduced.

Wilson and Jungner in the 1960's assessed the criteria which need to be addressed before embarking on a screening programme, including information about the disease itself, the test to be employed and the economics involved and their criteria have been adopted by the World Health Organisation. The disease should be reasonably common and serious and its natural history should be known. It should have a latent/symptomatic period during which time it would be appropriate to screen, treatment is available and treatment at the time of diagnosis should improve prognosis. Facilities for diagnosis should be in place prior to establishing the screening programme. There should be agreement on who to treat as a case and established treatments should exist for patients found to have the disease. The test should be valid and acceptable to patients and the cost of screening should be economically balanced in relation to expenditure on medical care as a whole.

This study so far has concentrated on the tests available and their ability to pick up endometrial abnormalities to validate the screening tools. It has been established that there is no ideal screening modality. A screening tool is not necessarily the best investigation available, which many would regard to be hysteroscopy, because hysteroscopy has limitations when considering its use in mass screening in both economic terms (equipment, operator time) and acceptability to patients. TV USS in contrast is well tolerated and fulfills a number of the other criteria as a screening tool but it has been shown in this and other studies to have a high false positive rate thus subjecting large numbers of asymptomatic women to more invasive procedures [Bertelli 1998, Neven 1994(a), (b), Langer 1997, Cecceni 1995, Lahti 1993, Cohen 1993(b)].

This study failed to find any significant abnormalities in a large group of asymptomatic women raising doubt about the appropriateness of introducing a screening programme/regular gynaecological review for women on long term tamoxifen which others have suggested [Ross 1995, Litta 1995, Bisset 1994, Barakat 1995, 1996, Dew 1995, Daniel 1996, Cuenca 1998].

Concentrating on the disease itself and what screening would achieve, it is known that endometrial cancer is slow growing and unlike ovarian cancer, tends to present early with vaginal bleeding. Because it presents early it is usually superficial and well differentiated, of low grade and early stage at diagnosis and therefore is associated with a good prognosis with an overall 80% 5 year survival (compared to 75-79% for breast cancer and 25% for ovarian cancer). It has been suggested in isolated reports that women who develop endometrial cancer on tamoxifen are more likely to develop high grade, poorly differentiated tumours [Assikis 1996, Altaras 1993, Hardell 1988, Seoud 1993], supporting the case for screening, but whole world literature does not confirm this.

What one aims to achieve from screening by picking disease up earlier than it would present clinically is to reduce the number of deaths from the disease. Breast cancer and cervical cancer screening achieve this because they pick up the disease during its latent/asymptomatic period. It is debatable whether endometrial screening can do this because it usually presents clinically early in its evolution with vaginal bleeding. It also does not seem economical to screen all women on tamoxifen using TV USS or any other

available tool to detect 1 extra endometrial cancer/1,000 women/year. This does not compare well with the 6/1000 pick up of breast screening.

Taking all these factors into consideration it can be concluded from this part of the study that screening asymptomatic women on tamoxifen is not indicated. Women on tamoxifen should be educated about the risks of this drug and be advised to report any symptoms in the form of abnormal bleeding/discharge for prompt investigation.

Ovarian Pathology

There is limited evidence that like the association between breast and endometrial cancer there is a positive association between breast and subsequent ovarian cancer again reflecting common risk factors [Ewertz 1990]. The additional effect of tamoxifen on the ovary is unclear. This study reported no difference in the incidence of ovarian cysts with tamoxifen use, however, others have found a significantly high incidence of functional cysts associated with tamoxifen use in premenopausal women compared with controls, most of which resolved spontaneously [Shushan 1996, Cohen 1999(a)].

Most agree that small unilocular cysts are best managed conservatively as they have a low incidence of malignant potential and only large complex cysts be surgically removed to exclude malignancy. Cohen et al [1996(b)] in a small study, suggested that postmenopausal women may be more susceptible to ovarian malignancy with tamoxifen use, however, numbers were small and other reports sporadic therefore this has yet to be confirmed [Hochner Celnikier 1995, Carter 1991]. The current study, nor that of Cook et all [1995] or Lindahl et al [1997] would confirm this finding.

Other Investigative Tools

This study concentrated on the relative merits and pitfalls of ultrasound scanning, hysteroscopy and pipelle biopsy as investigative tools for assessing the endometrium of women on tamoxifen. However, these are not the only tools which have been suggested to assess these women.

Saline infusion sonography (SIS) has been suggested as a technique which is less invasive than hysteroscopy but gives more information regarding the endometrial cavity than ultrasound scanning along. It involves inserting a narrow catheter into the uterine cavity and injecting a small amount of fluid into the cavity prior to performing the ultrasound scan. The catheter remains in situ during the scan in order for further fluid to be instilled into the cavity during the procedure if required to enhance the view [Goldstein 1996]. Some have advocated that the changes seen in the endometrium of women on tamoxifen on normal ultrasound scanning can be enhanced using SIS negating the need for further more invasive investigations. Goldstein [1996] reported five patients with bizarre heterogenous uterine changes on scan who underwent hysteroscopy and revealed smooth, atrophic endometrium. These patients were then viewed with SIS and the changes thought to be endometrium were shown to be located in the proximal myometrium, giving an explanation for the lack of tissue obtained at hysteroscopy. He does not extrapolate from this whether, if SIS had been performed prior to hysteroscopy, no further invasive investigation would have been performed. Others have suggested that SIS is useful for identifying polyps and submucous fibroids and therefore gives a more accurate measurement than of endometrium alone [Fleischer 1997, Karlsson 1994, Cohen 1995(b)] however this would not negate the need for further investigation in most women on tamoxifen as thickened endometrium still requires biopsy and polyps may still require removal. Some authors suggest the need for further assessment of SIS in tamoxifen users but accept its limitations at present [Cohen 1999, Barakat 1995, Karlsson 1994].

Magnetic Resonance Imaging (MRI) has also been studied as an investigative tool for women on tamoxifen. Ascher et al [1996] studied 35 postmenopausal women and found 2 distinct endometrial patterns, the first of which was homogenous and in keeping with atrophic or proliferative endometrium on histology. Ascher suggests that MRI may be

helpful in determining which patients require endometrial sampling but in view of the cost involved this does not seem to be a feasible screening tool.

CESSATION OF TAMOXIFEN

Introduction

Having established that tamoxifen causes apparent endometrial thickening on ultrasound scan and that this is related directly to the length of time a woman takes tamoxifen, it is important to establish whether this thickening is reversible following cessation of tamoxifen.

Although it is unknown why tamoxifen causes this scan appearance, it is likely to be due to endometrial serosal or superficial myometrial oedema and therefore it may be reversible after stopping tamoxifen.

The policy in Edinburgh until recently was to continue tamoxifen until the time of first recurrence, but following studies questioning the benefits of adjuvant tamoxifen for periods longer than 5 years, the majority of women who had been on tamoxifen for over 5 years stopped the drug following assessment of their endometrium. This presented an opportunity to study the effect of cessation of tamoxifen on the endometrium.

Patients and Methods

20 postmenopausal women (age range 53-74 years) who had thickened endometrium on ultrasound scan but atrophic, oedematous or cystic endometrium at hysteroscopy were asked if they would be willing to return after a time interval off tamoxifen to have the transvaginal ultrasound scan repeated. Women approached to return for scan off tamoxifen were those who reported that they would not object to returning for a third time. They were asked immediately following their normal hysteroscopy and no women approached declined to attend for a repeat scan.

All women stopped their tamoxifen following hysteroscopy and were off tamoxifen for between 6 and 11 months (mean 8.75 months) prior to repeat ultrasound scanning. Duration of tamoxifen use ranged from 61-148 months (mean 98 months) and ultrasound thickness on tamoxifen ranged from 6-23mm (mean 12.4mm).

Results

The results for these 20 women are summarised in Table 1.

At the second look ultrasound scan 16/20 (80%) had a reduction >2mm in endometrial thickness. This was a significant reduction at p=0.0013. Endometrial thickness prior to cessation of tamoxifen ranged from 6-23mm (mean 12.4mm, median 11mm, 95%C.I. 2.16) and following cessation of tamoxifen ranged from 4-15mm (mean 7.8mm, median 7, 95% C.I. 1.38)

This is demonstrated graphically in Figure 1.

Figures 2, 3 and 4 show changes in endometrial thickness following cessation of tamoxifen.

6/16 (38%) women whose endometrium reduced in thickness had an endometrium of normal thickness (<5mm) at second look ultrasound scan.

Of the 4 women whose endometrium remained unchanged or the reduction was <2mm (considered unchanged to allow for intra-observer error) 2 had only marginally thickened endometrium at the outset (7mm and 6mm) and the other 2 had endometrium measuring 10mm and 16mm. Both these women had normal or atrophic endometrium at hysteroscopy. There was nothing to distinguish these 4 women in terms of time on tamoxifen or length of time since cessation of tamoxifen, from the women whose endometrium decreased in thickness on ultrasound scan off tamoxifen.

Table 1

Details of women investigated following cessation of tamoxifen.

Patient	USS Thickness on tamoxifen	Hysteroscopy appearance	USS Thickness off tamoxifen mm	Time on tamoxifen (months)	Time off tamoxifen (months)
338.EH	15	N/atrophic	5	99	6
339.MR	16	N/atrophic	6	148	9
340.EC	11	cystic	9	121	8
341.MD	7	cystic	7	67	7
342.AM	6	N/atrophic	6	108	7
343.MM	7	N/atrophic	5	109	10
344.CM	19	N/atrophic	15	98	9
345.MD	16	N/atrophic	15	99	8
346.YH	9	N/atrophic	5	61	8
347.HS	8	N/atrophic	5	76	8
348.JP	10	N/atrophic	10	62	8
349.ED	23	N/atrophic	11	74	9
350.JF	15	oedematous	7	65	9
351.IW	11	N/atrophic	9	81	9
352.JH	19	N/atrophic	9	191	11
353.NT	11	oedematous	6	109	10
354.ES	8	N/atrophic	5	63	10
355.JK	12	cystic	9	62	11
356.RB	7	N/atrophic	4	123	9
357.MM	18	N/atrophic	8	144	9

Figure 1
Mean endometrial thickness on and off tamoxifen.

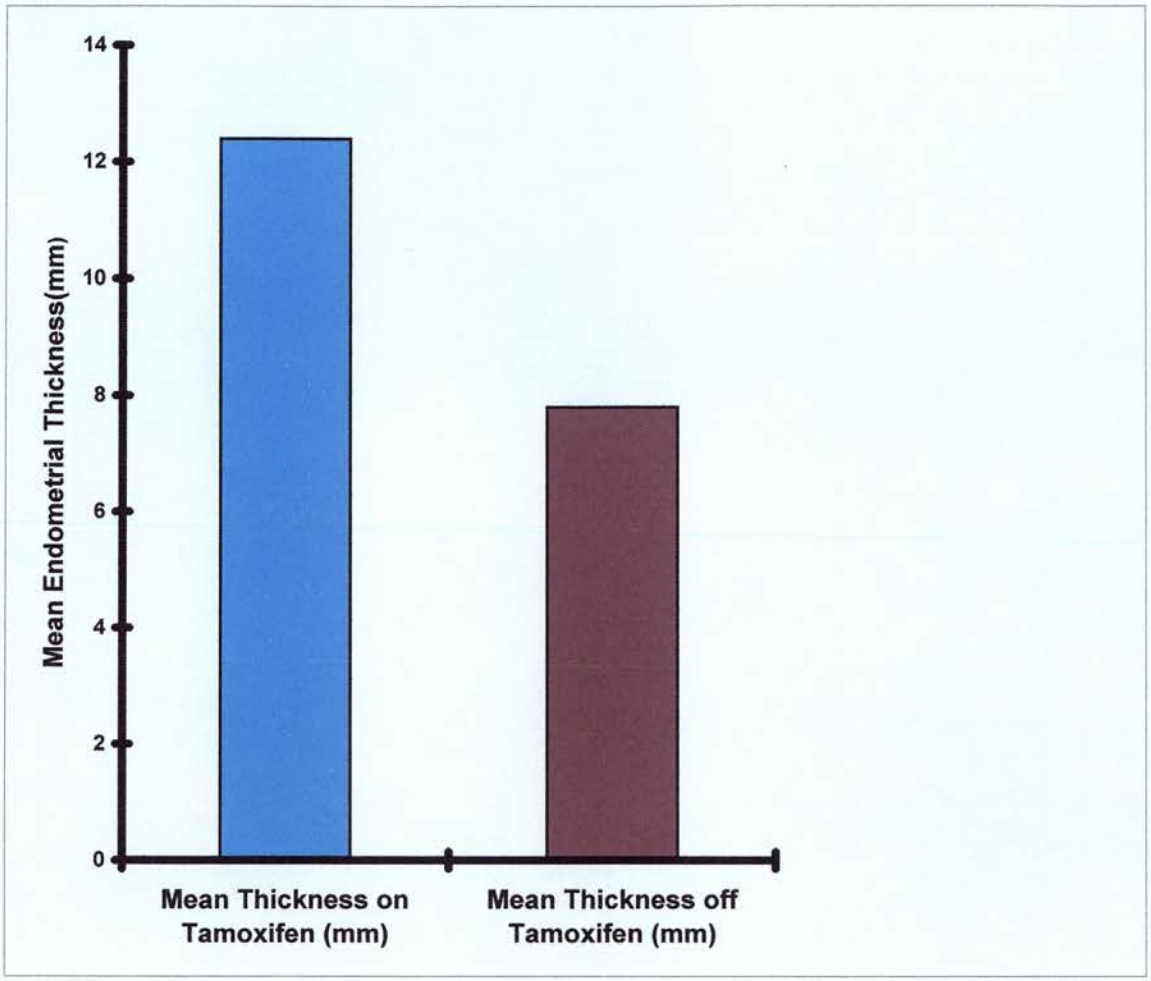
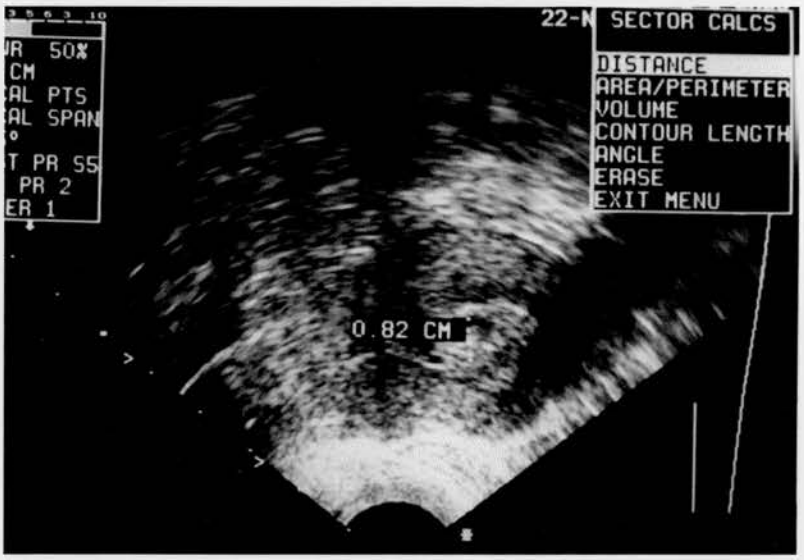


Figure 2

Reduction in endometrial thickness following tamoxifen cessation.

Figure 3

Reduction in endometrial thickness off tamoxifen.



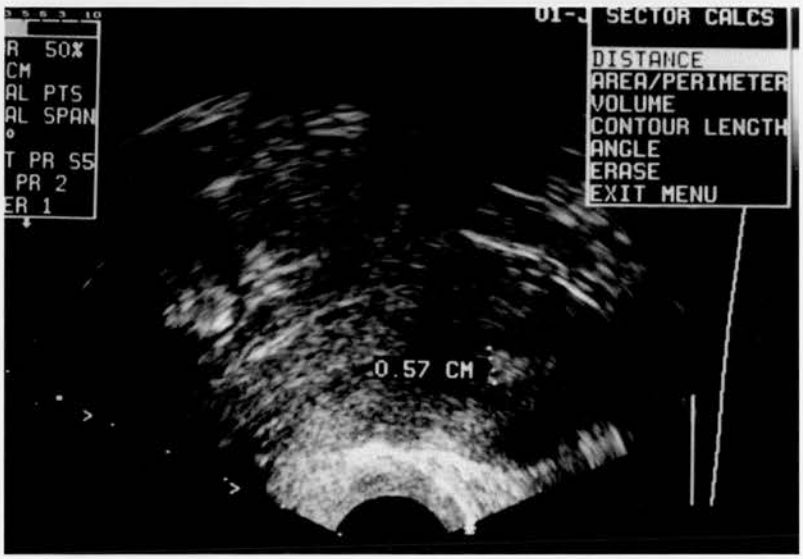
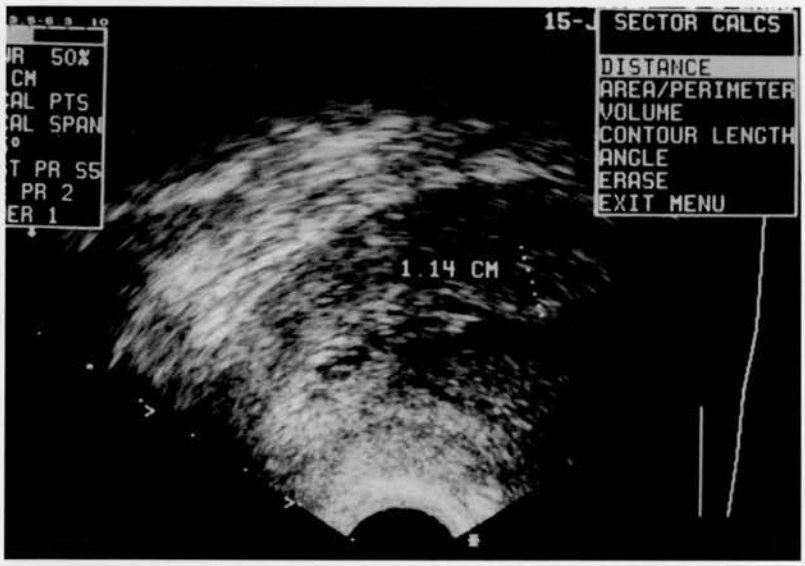


Figure 4

Reduction in endometrial thickness following tamoxifen cessation.



Discussion

The ultrasonographic endometrial appearances from women on tamoxifen are well documented. Anteby [1992(a)] described 4 women who had been on tamoxifen for 1-4 years and had an endometrial thickness of 12-34mm. All were sympton free but he described ultrasonographically a thick irregular endometrium containing cystic areas which were compatible with endometrial hyperplasia/neoplasia. However, at hysteroscopy, all had atrophic endometrium. He suggested that rather than this being purely endometrium on USS, tamoxifen was affecting the myometrium in some way to produce this false sonographic effect. Achiron [1995(a)] described the ultrasound findings as 'a peculiar endometrial honeycombe appearance' - abnormal endometrial thickening with multiple small cystic structures and heterogenous hyperechogenicity and more recently Neven [1998(a)] referred to the effect as 'Gruyere-cheese appearance'. Other workers have described similar cases where thickened endometrium is present on ultrasound scan but at biopsy atrophic rather than the expected hypertrophic endometrium the scan predicted was found [Aleem 1995, Ciatto 1994, Neven 1995, Goldstein 1994, Bornstein 1994, Cecchini 1996].

There have been a number of hypotheses regarding this.

Aleem [1995] suggested that the transvaginal ultrasound picture may represent small subendometrial sonolucencies in the proximal myometrium. Cohen [1993(b)] in a study of 72 asymptomatic women where there was no obvious correlation between the endometrial pathological findings and endometrial thickness assumed the discrepancy was due to echogenic cystic changes in the distal myometrium.

Ismail [1998(a)] regards this as a form of endometrial hyperplasia because the endometrium, although not technically abnormal, shows a low level of proliferative activity in epithelial and stromal cells, focal glandular crowding, endometrial stromal fibrosis and cystic dilatation of endometrial glands. Neven [1998(a)] argues that what is seen does not show clear endometrial proliferation or atypia and a similar picture has been classified as senile cystic atrophy in women not on tamoxifen. He believes that tamoxifen merely enhances the appearance of stromal fibrosis leading to cystically dilated endometrial glands.

While some refer to this as glandulocystic atrophy, he reclassifies this as pseudopolypoid glandulocystic endometrium or extensive senile cystic atrophy. It is clear from the literature that the pathology causing this apparent endometrial thickening remains under debate.

More recently, apparent endometrial thickening caused by tamoxifen has been related to the length of time on tamoxifen. The earlier findings in this thesis of 357 women on tamoxifen found this correlation to be highly significant and confirmed that thickening on ultrasound scan does not necessarily indicate endometrial pathology in that the study showed a 46% false positive scan rate. This finding has been confirmed in 3 other studies. Bertelli [1998] showed that 54% of patients on TV USS had endometrium >5mm which often appeared as multiple irregular sonolucencies suggesting the presence of cysts. However, ultrasound findings did not correlate with biopsy results, where no abnormalities were found. Hann [1997] investigated endometrial thickness on USS and also found a direct correlation between endometrial thickness on USS and duration of tamoxifen treatment. 48% of scans revealed an endometrial thickness of 8mm or more (which was used as the normal cut off) and concluded that endometrial thickness showed no correlation with symptoms. Bese [1996] in a similar study, used 10mm as the abnormal endometrial cut-off and found that women on tamoxifen had significantly thicker endometrium on ultrasound than controls but that there was a discrepancy between the sonographic findings and histology.

Although initially workers suggested that ultrasound scanning was the best tool for investigating women on tamoxifen and that all cases of thickened endometrium required further investigation, more recently some have questioned the role of ultrasound scanning in women on tamoxifen because of the high false positive results and the effect of tamoxifen duration on endometrial thickness as assessed by ultrasound [Hulka 1993, Love 1999, Bertelli 1998, Bornstein 1994]. Most now agree that USS thickness does not correlate with pathological findings and that this effect appears to be unique to tamoxifen use.

Tamoxifen is known to stimulate endometrial growth in some women and it is accepted that tamoxifen use is associated with an increased incidence of endometrial abnormalities,

including cancer. However, from knowledge about tamoxifen to date, it is impossible to predict from thickened USS appearances who is going to have significant pathology, benign or otherwise, from those who have atrophic/normal endometrium. This requires further research because the use of ultrasound would subject 46-58% of women, who we know from hysteroscopy and biopsy do not have significant pathology, to have unnecessary invasive procedures.

Whilst some workers have screened women with ultrasound prior to commencing tamoxifen [Berliere 1998, Tomas 1995, Willen 1998] and have monitored increasing endometrial thickness over time, no one has investigated women following cessation of tamoxifen to establish whether this phenomenon resolves.

The 20 women studied all had thickened endometrium on USS on tamoxifen with a range of 6-23mm (mean 12.4mm) and had a mean duration of tamoxifen use of 98 months. Following cessation of tamoxifen over a time period of 6-11 months there was a significant reduction in endometrial thickness from a mean of 12.4mm on tamoxifen to a mean of 7.8mm off tamoxifen ($p=0.0013$).

16/20 of the women studied had a reduction in endometrial thickness of >2mm suggesting that in these women, the tamoxifen effect is reversible and it could be postulated that if followed over a longer time period the endometrial thickness would revert to normal. In this group of 16 women over the period studied, although all had a reduction, only 6 fell to within normal limits (<5mm).

The remaining 4/20 women did not have a significant decrease in endometrial thickness. In 2 of these women the endometrium was only minimally thickened at the outset but the other 2 had endometrium measuring 10mm and 16mm. There was no obvious explanation why in these patients endometrial thickness did not decrease. It may be possible that follow-up was not long enough to identify a significant reduction.

A further hypothesis is that tamoxifen may have been stopped prior to pathology developing in these women with thickened endometrium on ultrasound scan. This would support a progressive effect theory where in some women the endometrium is never affected by tamoxifen (and there are some women who are on tamoxifen long term whose endometrium is normal both ultrasonically and hysteroscopically), some develop apparent thickening on ultrasound scan but are hysteroscopically and histologically normal but potentially, if left unchecked, develop into pathology - benign or otherwise. Supporting this theory is the fact that polyps which develop on tamoxifen often have glandulocystic elements [Corley 1992, Nuovo 1989]. Ismail [1994] has also suggested that polypogenesis may form an important intermediate stage between simple hyperplasia and malignancy and that there may be a dose threshold of tamoxifen for this to occur.

Normal <———> Thickening ———>Thickening ———> Pathology
(reversible) (non reversible) Benign——> Malignant

The reduction in thickness in some women with cessation of tamoxifen suggests that whatever is responsible for the apparent endometrial thickening is in most women reversible. Anecdotally, Rutqvist (1997) reported recently an update from the Stockholm trial which showed that the risk of developing endometrial abnormalities did not appear to reduce following cessation of tamoxifen. Potentially, Rutqvist may be studying women with irreversible changes which may have been present in 4 of our 20 women.

The other interesting group of women who have been ignored by investigators to date is the group whose endometrium is never affected by tamoxifen regardless of length of time on the drug. Clearly there is some as yet undiscovered trigger which does not affect these women. These women may be at a lower risk of endometrial cancer and an understanding of why these women's endometrium is unaffected by tamoxifen would be a significant advance in our knowledge of the tamoxifen/uterine interaction.

ENDOMETRIAL PATHOLOGY

Introduction

Tamoxifen has been shown to cause increased endometrial thickening on ultrasound scan in 41% of asymptomatic women studied. On further investigation, (out-patient hysteroscopy), 46% of these women had normal or atrophic endometrium and the remainder all had benign features in the form of polyps or submucuous fibroids to account for the scan appearances. There have been several hypotheses to account for this apparent endometrial thickening but the histology remains unclear.

One of the aims of this thesis was to investigate this false positive scan appearance histologically to establish a cause for this finding. As endometrial biopsies were to be taken from all thickened endometria at hysteroscopy for histology, a second aim was to study markers of cell proliferation, ER and MIB 1, to assess the effect of tamoxifen on endometrial cell turnover.

Unfortunately, despite numerous attempts to biopsy the endometrium of women at hysteroscopy using various biopsy forceps and Sharman Endometrial Biopsy Curette, (Dupae Health Care, Leeds, UK) insufficient samples were obtained. Several other workers have reported the fact that tamoxifen treated endometrium is difficult to biopsy even in the presence of ultrasonographic abnormalities but the reason for this is yet to be established.

Therefore in order to obtain sufficient samples from tamoxifen treated women and controls, pathological specimens from hysterectomised women were retrieved and studied.

Patients and Methods

195 women were identified as previously described ***Methods*** : ***Tissue Specimens***. In 134 cases, medical records were retrievable and were investigated and from these 48 women were identified as being on tamoxifen at the time of their hysterectomy and 19 women identified to act as controls. Only women who had hysterectomy performed for benign pathology were included.

Pathology specimens from 34 women on tamoxifen and 14 controls were traced and suitable for investigation. 47 of the specimens were hysterectomy specimens and one was an endometrial polyp (the hysterectomy specimen in this patient was not traceable). In several patients there were D & C specimens (endometrial curettings) available in addition to the hysterectomy specimen but these were not investigated. Reasons for hysterectomy included menorrhagia/dysfunctional uterine bleeding, fibroid uterus, uterine prolapse, ovarian cyst and postmenopausal bleeding (no malignant cause identified).

The age range of women on tamoxifen was 44 - 73 years (mean 58 years) and that of controls 34-79 years (mean 53 years).

7 of the tamoxifen users were premenopausal and 5 of the controls at the time of hysterectomy.

Histology

Representative haematoxylin and eosin (H&E) endometrial slides from each patient were identified for investigation. Following receival of surgical specimens by the pathology department, tissue blocks from these specimens are cut and from these, 4μm sections are cut, mounted and stained (H&E) for histological investigation. Therefore for each specimen received there are several tissue blocks and H&E slides. One slide showing a representative section of endometrium and myometrium was chosen from each patient for the purposes of this study.

Each slide was then assessed blindly by one investigator (Dr Alistair Williams, Consultant Pathologist, Edinburgh Royal Infirmary) studying various aspects of the endometrial glands and stroma and the interface between endometrium and myometrium. All information was recorded onto a histology proforma for further assessment.

Immunohistochemistry

A sub-group of the study population, 16 women on tamoxifen and 5 controls, had further tissue sections cut and stained to investigate markers of cellular proliferation, ER and MIB 1. The methodology was described in ***Methods : Immunohistochemistry.***

These sections were thereafter blindly assessed simultaneously by two investigators, the author and Dr Margaret McIntyre, Consultant Pathologist, Western General Hospital, Edinburgh. Each slide was scored semiquantitatively on the proportion of cells staining positive for ER and MIB 1 and the intensity of the staining.

Proportion Score (PS) ranged from 0-5:	Cells Staining
0	0
1	1/100
2	1/10
3	1/3
4	2/3
5	1
Intensity Score (IS) ranged from 0-3:	Intensity of cells staining
0	0
1	weak
2	intermediate
3	strong

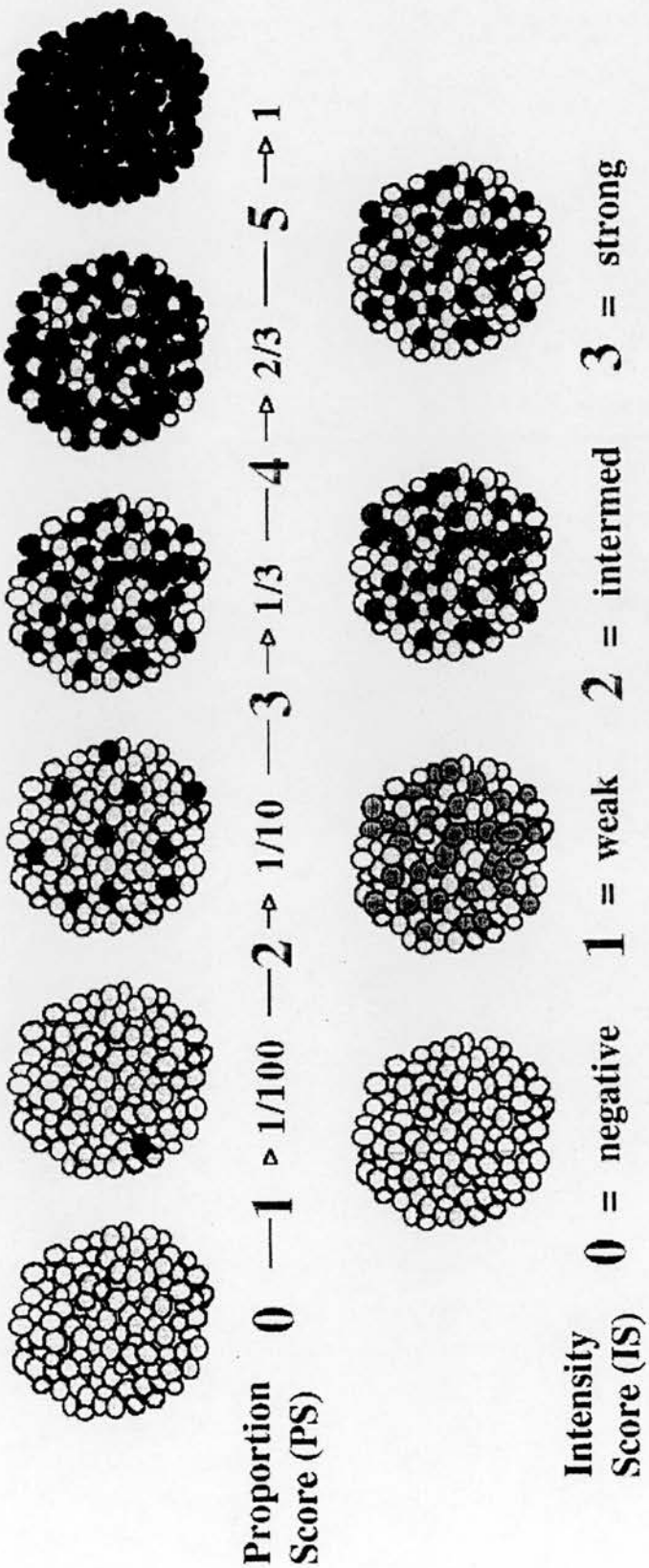
For each slide the endometrial glands and stroma were scored separately for ER and MIB1 as the glands and stroma appeared to stain to differing degrees making a single score inaccurate.

A Total Score = PS + IS was calculated for each modality.

The scoring procedure is illustrated in Figure 1.

Figure 1

Immunohistochemistry scoring procedure.



Results

Histology

From the histological evaluation of the endometrial glands and stroma the results from the 34 tamoxifen treated patients and 14 controls are summarised in Tables 1 and 2.

The factors which were significantly different between tamoxifen users and controls were;

1. cystic dilatation of endometrial glands p=0.012
2. pattern of stromal cellularity p=0.012
3. quantity of stromal collagen p=0.011

Tamoxifen users were significantly more likely to show glandular cystic dilatation, low stromal cellularity and the presence or prominence of collagen within the endometrial stroma. None of the other factors investigated reached statistical significance.

Figure 2 shows an H&E slide from a tamoxifen user with the typical cystic dilatation and Figure 3 an H&E slide showing collagenous endometrial stroma.

The mean age of tamoxifen users was 58 years and that of controls 53 years which was not significantly different. This is of importance because menopausal state may have been an important factor in tamoxifen's histological effect on the endometrium.

Table 1

Endometrial glandular histology from tamoxifen users and controls.

	Tamoxifen	Controls	
Glands	(34)	(14)	
Cystic Dilatation			
0	4	7	p=0.012
Mild/Focal	8	3	
Prominent/Widespread	22	4	
Focality			
Heterogeneous	13	2	
Homogeneous	21	12	
Profiles			
Straight	18	6	
Sinuous	8	4	
Coiled	7	2	
Tortuous	1	2	
Arrangement			
Widely spaced	19	4	
Normally spaced	13	10	
Crowded	2	0	
Activity of Cells			
Flat	7	3	
Columnar	27	11	
Mitotic Activity			
Absent	27	12	
Low	5	2	
Significant	2	0	
Nuclear Atypia			
Absent	32	12	
Mild	2	2	
Significant	0	0	

Table 2

Endometrial stromal histology from tamoxifen users and controls.

	Tamoxifen	Controls	
Stroma	(34)	(14)	
Cellularity			
Heterogeneous	31	14	
Homogeneous	3	0	
Significant Pattern			
Low cellularity	12	1	p=0.012
Normal	16	13	
Condensed	6	0	
Oedema			
Absent	28	9	
Present	5	5	
Collagen			
Absent	7	9	p=0.011
Present	11	3	
Prominent	16	2	
Vessels			
Thin walled	24	10	
Thick Walled	10	4	
Interface			
Sharp	12	9	
Adenomyotic	22	5	

Figure 2

H & E slide showing endometrial cystic dilatation secondary to tamoxifen use.

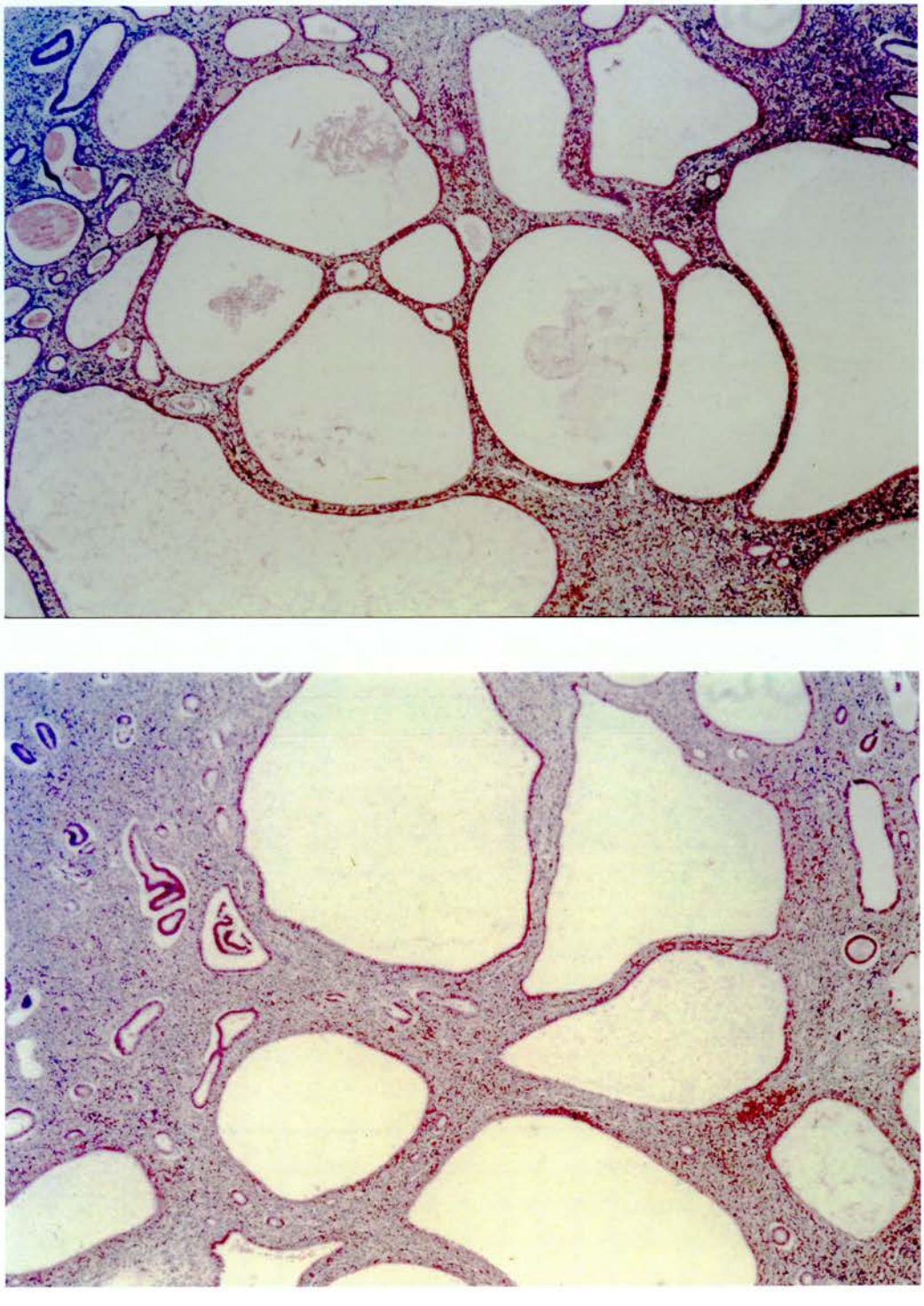
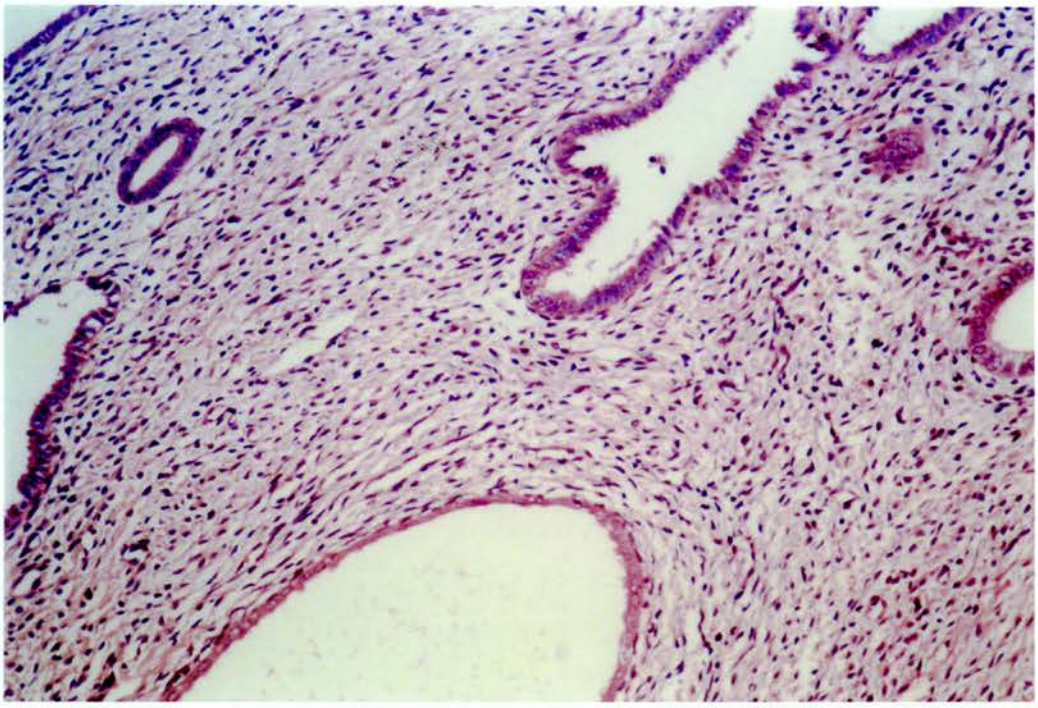


Figure 3

H&E slide showing collagenous endometrial stroma in a tamoxifen user.



Immunohistochemistry

The tissue block from which the H&E slide assessed was taken was identified and further sections cut, mounted and stained as previously described to assess markers of cell proliferation ER (oestrogen receptor) and MIB 1.

The results for ER and MIB 1 staining for tamoxifen users and controls are in Tables 3, 4, 5 and 6 respectively.

In the tamoxifen group there were 16 patients - 4 premenopausal and 12 postmenopausal, and in the control group 5 patients - 2 premenopausal and 3 postmenopausal. There was no significant difference in ER or MIB 1 expression between pre and postmenopausal patients in either the tamoxifen or control group.

The glandular cells of the endometrium stained positive for ER in all tamoxifen users and all controls and the stromal cells positive for ER in 12/16 (75%) of tamoxifen users and 3/5 (60%) of controls. Glandular cells were positive for MIB 1 in all tamoxifen users and 60% (3/5) of controls and stromal cells for MIB 1 in 50% (8/16) of tamoxifen users and 80% (4/5) controls.

There was no significant difference in expression of ER or MIB 1 between study and control patients for endometrial glands or stroma. The MIB 1 staining in endometrial stroma almost reached statistical significance ($p=0.073$) indicating that tamoxifen users were less likely to stain positive for MIB 1 than controls. Table 7 shows mean and median scores for ER and MIB 1 in tamoxifen users and controls.

Figures 4 and 5 show staining for ER and MIB 1 in tamoxifen users and Figures 6 and 7 staining for ER and MIB 1 in controls.

Table 3

Immunohistochemistry ER results from tamoxifen users.

Pathology No	Glands			Stroma		
	PS	IS	Total	PS	IS	Total
26456/91	4	3	7	4	3	7
25138/91	5	1	6	-	-	-
11151/91	4	1	5	4	1	5
7080/92	5	3	8	-	-	-
6946/92	5	1	6	3	1	4
1515/93	4	2	6	4	2	6
8686/93	5	3	8	4	3	7
19132/93	5	2	7	-	-	-
22436/93	5	2	7	4	2	6
14851/95	4	1	5	4	1	5
14002/95	4	1	5	4	2	6
5020/95	3	1	4	4	1	5
12995/95	5	2	7	4	2	6
23681/94	5	2	7	3	2	5
17418/94	5	2	7	-	-	-
13779/94	5	2	7	1	1	2

Table 4

Immunohistochemistry ER results from control patients.

Pathology No	Glands			Stroma		
	PS	IS	Total	PS	IS	Total
10065/92	5	3	8	-	-	-
17864/92	3	1	4	1	1	2
22957/92	5	3	8	3	2	5
26014/94	3	1	4	-	-	-
5554/95	5	3	8	3	2	5

Table 5

Immunohistochemistry MIB 1 results from tamoxifen users.

Pathology No	Glands			Stroma		
	PS	IS	Total	PS	IS	Total
26465/91	3	2	5	-	-	-
25138/91	1	2	3	-	-	-
11151/91	1	2	3	1	2	3
7080/92	3	3	6	1	2	3
6946/92	1	3	4	1	3	4
1515/93	2	3	5	1	3	4
8686/93	3	3	6	1	3	4
19132/93	1	2	3	-	-	-
22436/93	1	3	4	1	1	2
14851/95	1	3	4	-	-	-
14002/95	1	3	4	-	-	-
5020/95	1	3	4	-	-	-
12995/95	1	3	4	1	3	4
23681/94	1	3	4	1	3	4
17418/94	3	3	6	-	-	-
13779/94	1	5	6	-	-	-

Table 6

Immunohistochemistry MIB 1 results from control patients.

Pathology No	Glands			Stroma		
	PS	IS	Total	PS	IS	Total
10065/92	1	3	4	-	-	-
17864/92	-	-	-	3	3	6
22957/92	2	3	5	1	3	4
26014/94	-	-	-	2	3	5
5554/95	1	3	4	1	3	4

Table 7

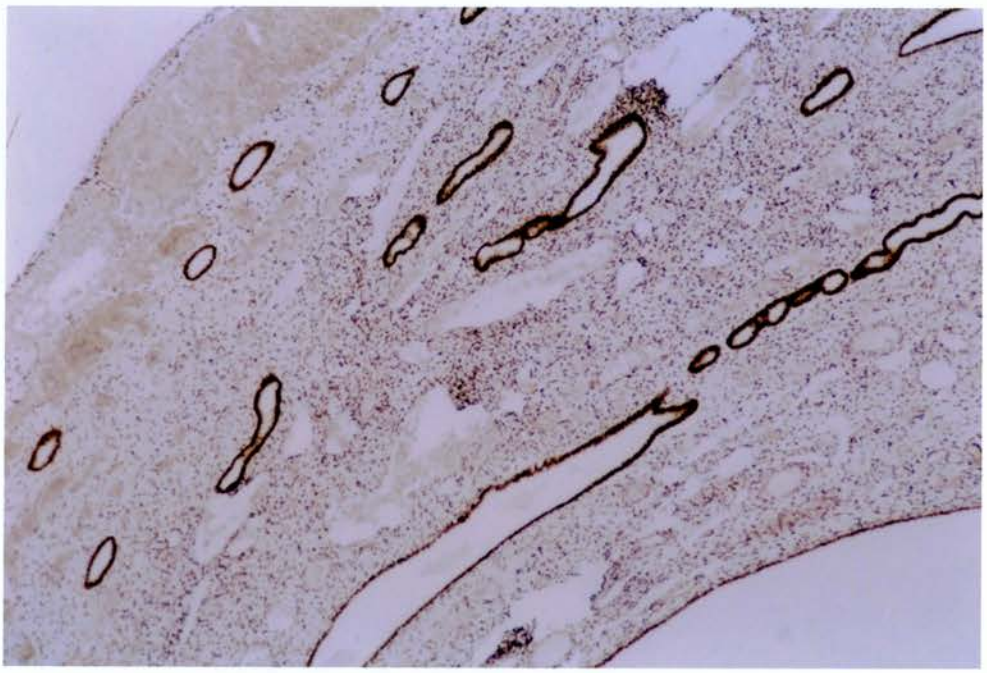
Mean and median scores for ER and MIB 1 from tamoxifen users and controls.

	Mean	Median	
ER			
Glands			
Tamoxifen	6.4	7.0	p=0.66
Controls	6.4	8.0	
Stroma			
Tamoxifen	4.0	5.0	p=0.21
Controls	2.4	2.0	
Total (Glands + Stroma)			
Tamoxifen	10.4	10.0	P=0.45
Controls	8.8	8.0	

	Mean	Median	
MIB 1			
Glands			
Tamoxifen	4.4	4.0	p=0.21
Controls	2.6	4.0	
Stroma			
Tamoxifen	3.5	4.0	p=0.073
Controls	4.8	4.5	
Total (Glands + Stroma)			
Tamoxifen	6.2	6.0	p=0.84
Controls	6.4	6.0	

Figure 4

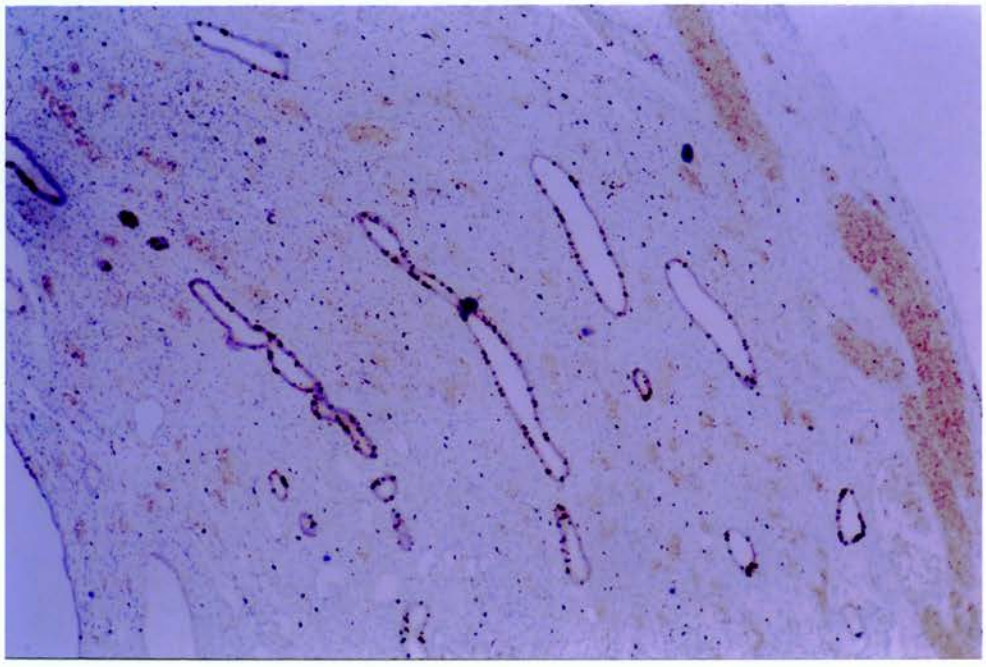
ER staining from tamoxifen user.

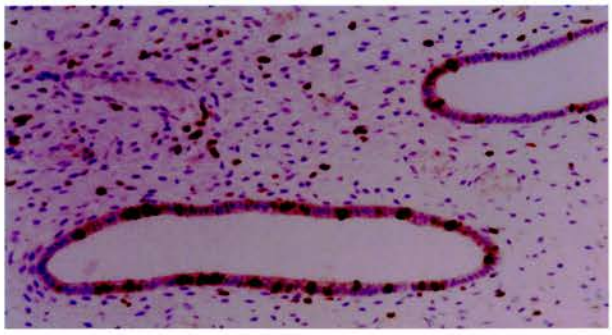


Score : Glands (5+2) 7 Stroma (3+2) 5

Figure 5

MIB 1 staining from tamoxifen user.

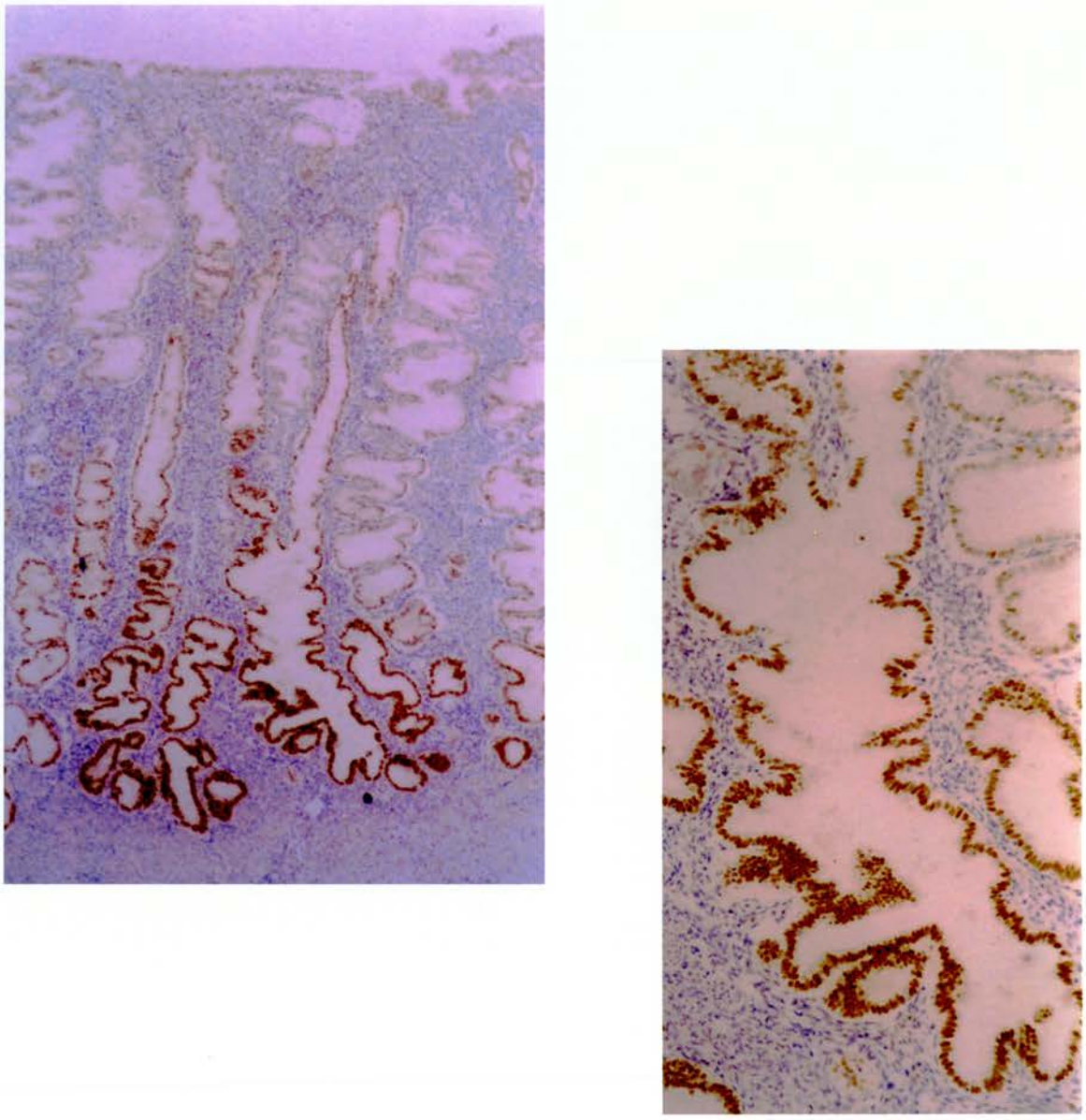




Score : Glands (2+3) 5 Stroma (1+3) 4

Figure 6

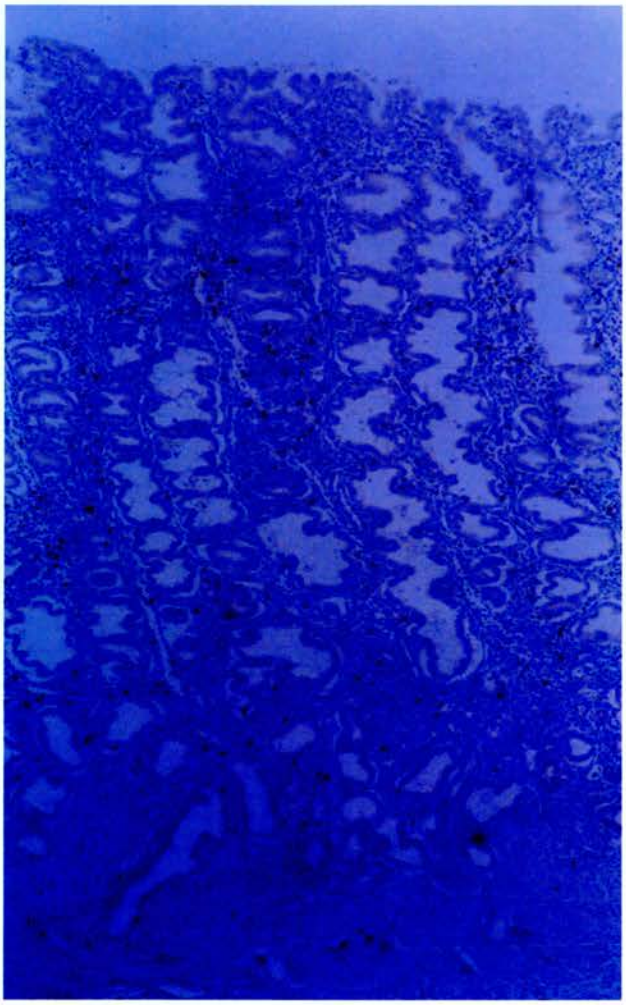
ER staining from control patient.



Score : Glands (3+1) 4 Stroma 0

Figure 7

MIB 1 staining from control patient.



Score : Glands 0 Stroma (2+3) 5

Discussion

Histology

The histology responsible for apparent endometrial thickening on ultrasound scan seen in women on tamoxifen from current literature is uncertain. This effect has been noted as far back as 1992 when Anteby et al described 4 women who had endometrial thickening on ultrasound scan but atrophic endometrium at hysteroscopy in the presence of this ultrasound finding and has been confirmed by several other groups since then [Anteby 1992].

It is now accepted that tamoxifen treated endometrium can be very difficult to biopsy even in the presence of ultrasonographic abnormalities and this has been described as 'endometrial honeycomb appearance' [Achiron 1995 (a)] and 'Gruyere-cheese appearance' [Neven 1998(a)]. In addition, it is usually not possible to predict what the histology will be from the ultrasonographic appearances.

Hulka [1993] described multiple sonographic appearances including hyperechoic, homogenous tissue, hyperechoic tissue with multiple small cystic spaces, heterogenous tissue with small cystic spaces and solid heterogenous tissue in a group of 11 patients undergoing sonography. She concluded that, because several pathologic findings could coexist in tamoxifen patients, making it difficult to assign a single ultrasound feature to a single pathologic diagnosis, ultrasound was of limited value.

Bornstein [1994] studied 22 tamoxifen patients, 7 symptomatic and 15 asymptomatic, all with ultrasound abnormalities - irregular thickening of the endometrium, some with cystic formations. He found no pathology or inadequate material for assessment from all 15 asymptomatic patients and polyps in 2/7 symptomatic women. He suggested that the discrepancy between ultrasound and histology may be due to stromal oedema which was insignificant and not a true abnormality. In view of these findings he also questioned the role and need for screening.

Touraine [1995] described 40 women on tamoxifen who underwent hysteroscopy. 22 had previously had ultrasonography performed and in 20 women 'hyperplasia' had been

diagnosed. At hysteroscopy all 40 women had 'a smooth, white and brilliant endometrium associated in most cases with multiple scattered protuberences.' In 16 of these women biopsy was not possible because of the 'intensity of atrophy' and the remaining 24 all had atrophic endometrium on histology. He described dilated cystic glands on histology, with a flattened epithelium but a dense stroma with an abundance of collagen and large oedematous areas. Mitoses were uncommon and he described the whole picture as cystic glandular atrophy.

Achiron et al [1995 (a), (b), 1996] described thickened, irregular cystic endometrium on ultrasound scanning in 20 postmenopausal women on tamoxifen, 8 of whom had polyps and the remaining 12 no significant pathologic findings - scanty, senile, cystic atrophic endometrium. Both Achiron and later McGonigle [1998] suggested that this appearance may be better investigated by sonohysterography as this may distinguish between polyps and 'cystic atrophy', the polyps requiring removal as the overall incidence of endometrial polyposis in the tamoxifen population ranges from 39-50% and some have suggested may be a precursor to hyperplasia or carcinoma. Achiron concluded that tamoxifen stimulates the endometrial epithelium and stroma and together with the myometrium may cause the thick echogenic honeycomb appearance without necessarily causing epithelial disease.

Willen [1998] prospectively followed 29 patients on tamoxifen. All had ultrasound prior to commencing tamoxifen and at regular intervals thereafter. Where endometrial thickness was >5mm at any point in the screening programme they underwent histological evaluation. His group reported proliferation of stromal cells with slight hyperchromasia and definite increased cell density. In general the epithelium was described as atrophic with polyp formation but sometimes showed features of proliferation with mitoses, or cytoplasmic 'snouts' bulging into the lumen. The glands also demonstrated cystic dilatation.

McGonigle et al [1998] studied 35 tamoxifen treated women with endometrial thickness >5mm on ultrasound scan. 23/35 patients were found to have polyps accounting for their thickened endometrium but 10/35 (29%) had endometrial cystic atrophy to explain the USS findings. This was defined as multiple cystic spaces lined by atrophic epithelium present within a dense fibrous stroma. Changes were often seen extending to the endomyometrial junction. However, ultrasonographic findings did not distinguish between the presence of

endometrial polyps or cystic atrophy and concluded that cystic atrophy was probably a benign process unlikely to cause an increased risk of endometrial cancer.

Bertelli et al [1998] studied 110 asymptomatic patients on tamoxifen and found that 54% had an endometrial thickness of >5mm, often with multiple irregular sonolucencies suggestive of cysts, but that ultrasound findings did not correlate with abnormalities at biopsy and therefore questioned the use of sonography in asymptomatic women. He suggested that the tamoxifen induced hyperproliferation may be mediated through the endometrial stroma in that hypertrophy of the sub endometrial stroma was seen histologically in some of the biopsy specimens.

Mourits et al [1999] conclude that the histologic changes can be summarised as hyperplastic endometrial stroma and atrophic epithelium and this would explain the fact that several endometrial pathologies, atrophy, hyperplasia or polyps present with similar ultrasonographic findings.

Neven et al [1998(a)] report that in postmenopausal women not on tamoxifen histopathological evidence of flattened or cuboidal epithelium overlying fibrous endometrial stroma containing cystic dilated endometrial glands is not rare and is usually referred to as senile cystic atrophy but he believes that tamoxifen greatly enhances this appearance of stromal fibrosis leading to cystic dilated endometrial glands. He suggests that referring to the appearance as hyperplastic is misleading and reclassifies it as pseudopolypoid glandulocystic endometrium or extensive senile cystic atrophy.

Ismail [1994(a), 1995, 1998(a)(b), 1999] has published widely on this topic reporting macroscopic 'Swiss cheese like cut surface' with generalised thickening of the endometrium within which are numerous cysts of varying size. Microscopically she finds cystic dilatation of the endometrial glands with some glands showing glandular budding, fibrotic endometrial stroma and collagen bundles separating stromal cells, the stroma being relatively 'paucicellular'. Tamoxifen induced polyps show patchy periglandular stromal condensation and a proliferation of epithelial and stromal cells. There appears to be no cytological atypia and minimal mitotic activity. Thus there is a spectrum of proliferative abnormalities and she suggests that this may form an important intermediate stage between

simple hyperplasia and malignancy. She attributes the problem of inadequate tissue sampling to endometrial stromal fibrosis and does not accept the term 'glandulocystic atrophy' as this implies normal atrophic endometrium. Ismail maintains that tamoxifen stimulated endometrium shows a degree of low proliferative activity in epithelial and stromal cells, focal glandular crowding, endometrial stromal fibrosis and florid cystic dilatation which is best regarded as a form of hyperplasia.

This lack of ability to satisfactorily biopsy the endometrium in these women led to the hypothesis that it may be myometrial in origin and represent superficial myometrial oedema.

Goldstein [1994] described a heterogenous bizarre ultrasonographic appearance in five women which he felt initially to be endometrial in origin but following saline infusion (sonohysterography) was thought to represent small subendometrial sonolucencies in the proximal myometrium and was reinforced by the fact that hysteroscopy revealed a smooth endometrium and histology, inactive endometrium. He concluded that this appearance may represent abnormal adenomatous-like changes in the proximal myometrium. Cecchini [1996] and Cohen [1993(a), 1994(a)(b)] also detected endometrial thickening without abnormality and suggested that the changes may be myometrial because of the inability to obtain adequate biopsy specimens.

Summarising all the evidence to date, the appearance on ultrasound scan would suggest the thickening to be endometrial in origin, with a typically normal appearance at hysteroscopy which is difficult to biopsy. Transvaginal ultrasound does not differentiate between this and other endometrial pathologies making it of limited use. This effect appears to be unique to tamoxifen use, but its histological classification remains under debate.

It would have been ideal to have obtained adequate tissue specimens at hysteroscopy from the population of asymptomatic women studied to investigate the histology causing the apparent endometrial thickening but despite numerous attempts to do so in the out-patient setting this was found to be difficult, as previously described. Therefore it was necessary to use the second study population described.

This study, the first to use a control population to investigate histology, found that tamoxifen users had significantly more cystic dilatation of endometrial glands, low stromal cellularity but the presence or prominence of collagen within the endometrial stroma. These results confirm those of other workers apart from the degree of stromal cellularity which we found to be low while others have suggested stromal proliferation and dense stromal cellularity. These results would agree with suggestions that the quantity of collagen within the stroma could account for the difficulty in biopsying tamoxifen induced endometrium.

The significance of these endometrial findings is yet to be established.

Immunohistochemistry

Tamoxifen, effective in the treatment of breast cancer, is thought to down regulate oestrogen receptors (ER) in the breast by acting as an oestrogen receptor antagonist. However, tamoxifen acts as a partial agonist on the endometrium, and through mechanisms unknown is responsible in users for an increased incidence of endometrial abnormalities including cancer.

The aim was to investigate possible mechanisms of action further by assessing the expression of ER in the endometrium of tamoxifen users and controls to test the hypothesis that tamoxifens mode of action is to upregulate ER expression in the endometrium, giving some explanation for its agonist effects. In addition, MIB 1 was assessed in the same groups as a marker of epithelial proliferation to examine whether cellular proliferation was an important factor in the carcinogenic potential of tamoxifen.

In the normal population all endometrium has been shown to contain oestrogen receptors, with the highest values seen in the proliferative phase of the menstrual cycle and the lowest values in the secretory phase. Ehrlich et al [1981] showed that 93% of endometrial hyperplasia and 79% of endometrial cancer was positive for ER and suggested that the proportion of ER staining fell with increasing tumour anaplasia [Bayard 1979, Mortel 1981].

Kommoss et al [1998] in a retrospective study, evaluated the presence of ER in the endometrium of 40 tamoxifen users. They found positive ER staining in the glandular cells of all 40 women and weak to moderate ER staining was also observed in endometrial stromal cells. They detected ER positive cells in the endometrium of 16/20 control patients (80%) but concluded that the persistent finding of ER positive glandular cells in tamoxifen users supported its suspected oestrogen agonist effect. Gorodeski [1992] gave tamoxifen in a single dose and monitored ER for 4-96 hours post administration. He found that ER increased following tamoxifen, confirming agonistic properties and that the highest relative increase in ER levels were seen in menopausal women indicating that postmenopausal endometrium is sensitive to tamoxifen.

However, there have been varying reports regarding tamoxifen's effect on endometrial ER. Carlson et al [1984] investigated tamoxifen's use in the treatment of endometrial cancer when it was thought that tamoxifen was a pure antioestrogen and found that in 17 patients with endometrial cancer tamoxifen decreased ER expression in 9 but increased ER expression in the remaining 6 women possibly due to tumour heterogeneity and concluded that the trend was towards tamoxifen decreasing ER. Neumannova et al [1985] showed a depletion in cytosol ER and an increase in nuclear ER but a tendency towards a decrease in total cellular ER while Kokko et al [1981] suggested that tamoxifen administration did not alter ER concentration. Perez-Lopez [1993] showed that tamoxifen significantly reduced endometrial receptor levels compared to controls.

In the small population investigated in this study there was no significant difference in ER or MIB 1 expression in tamoxifen users or controls regardless of menopausal state.

All glandular cells in tamoxifen users did stain positive for ER and MIB 1 and this may reflect the histology seen with tamoxifen use, namely large cystically dilated glands which has been shown to be unique to tamoxifen use and indicates a degree of activity in the glandular elements of the endometrium. Histology investigated as part of this study revealed low stromal cellularity in tamoxifen users and this may be reflected in the fact that only 75% of stromal cells stained positive for ER and 50% for MIB 1 indicating a lower degree of stromal activity. The degree of stromal staining for MIB 1 was almost significantly less in tamoxifen users compared to controls ($p=0.073$).

Kuwashama [1996] found low positivity for ER in women on tamoxifen with endometrial cancer (1/7) and suggested that tamoxifen may not in fact operate through oestrogenic pathways. Other mechanisms of action of tamoxifen have included the formation of DNA adducts. In rat liver, tamoxifen is metabolised to alpha-hydroxytamoxifen, the proximate carcinogen, which is activated by further metabolism to a sulphate ester which gives rise to high levels of DNA adducts. Barakat et al [1998] found that in vivo DNA adduct formation in tamoxifen treated women did not differ from that in untreated patients and that there was no good evidence for in vivo DNA adduct formation [Powles 1995]. This has been supported by other workers in vivo and in vitro regardless of dose or length of treatment [Vergote 1998, Carmichael 1996, 1999].

Proto-oncogene expression in endometrium has also been suggested as a possible mechanism of action. In rat endometrium the persistent over expression of c-fos and jun-B has been demonstrated and the products of these cellular oncogenes (AP-1 transcription factor) are thought to play important roles in regulating cellular function in many tissues including uterus [Nephew 1996, Zhao 1998, Neven 1993 (a)]. Robertson [1998] found a similar up regulation of c-fos in ewes.

Tamoxifen can also induce chromosomal changes in rat hepatocytes and mutations of the tumour suppressor gene p53 in rat livers leading to hepatocellular carcinoma in rats. p53 is the most commonly mutated gene in human cancers and has been correlated with higher grade advanced stage, recurrence risk, metastases and more aggressive tumours. Ramondetta [1998] examined hysterectomy specimens from endometrial cancer in breast cancer patients on and off tamoxifen and found that tamoxifen associated tumours expressed p53 mutations more frequently than non users and that p53 mutations may play a role in the development of tamoxifen associated tumours [Carmichael 1999].

There have also been reports of an increased incidence of cancer foci developing in polyps associated with tamoxifen use. Dal Cin et al [1998] investigated genomic changes in endometrial polyps but found no evidence for tamoxifen induced chromosome changes or gene rearrangement to support this carcinogenic theory.

Other growth factors have also been postulated to play a role in tamoxifen associated endometrial tumours including IGF-1 and IGFBP (upregulation of), adrenomedullin (a growth factor for endothelial cells which is angiogenic) and TGF-B, although none convincingly [Fornander 1991, Zhao 1998, Laatikainen 1992, Nephew 1996, Kuo 1995, Ross 1995]. Thus far the cellular mechanism for tamoxifen's action on the uterus remains unknown.

TAMOXIFEN SIDE EFFECTS

Introduction

Tamoxifen is the most commonly prescribed adjuvant therapy for women with breast cancer and since its introduction in 1971 has been used by millions of women worldwide. As a chemotherapeutic agent it is extremely well tolerated with few serious toxic side effects apart from its effect on the endometrium.

However, women taking tamoxifen do report a number of less serious side effects, which, although not life threatening, can be troublesome and affect quality of life, eg hot flushes, night sweats and weight gain. Few studies have assessed the incidence of these side effects in women on long term tamoxifen and have compared them to a control population of similar age with breast cancer.

The aim was to assess the incidence of non-toxic side effects in the study group of 357 women on long term tamoxifen and 130 control women who had been treated for breast cancer but had not received tamoxifen to assess which side effects were significant.

Patients and Methods

All patients were asked to complete a side-effect questionnaire asking whether they were experiencing various symptoms including hot flushes, sweats, weight gain, fluid retention etc. Only current symptoms were included for analysis.

Results

Tables 1 and 2 give questionnaire results for women on tamoxifen and controls respectively.

All 357 women on tamoxifen and 130 controls completed the questionnaire. The results can be summarised as follows:

	Study Group	**Control Group**	
	357	130	
Hot flushes/sweats	210 (59%)	59 (45%)	p=0.0099
Weight gain	155 (43%)	47 (36%)	NS
Low energy	87 (24%)	36 (28%)	NS
Fluid retention	85 (24%)	15 (12%)	p=0.0033
Low libido	76 (21%)	28 (22%)	NS
Vaginal dryness	81 (23%)	32 (25%)	NS
Vaginal discharge	149 (42%)	16 (12%)	p<0.0001

This is demonstrated graphically in Figure 1.

The only side effects which were significant were hot flushes/sweats, 'fluid retention' and vaginal discharge.

Fluid retention is generally a subjective phenomenon and its significance should be interpreted with caution.

Although there appeared to be an increase in the percentage of women reporting weight gain on tamoxifen, this was not statistically significant.

Other side effects which women were questioned about (example questionnaire over) included nausea, irritability, skin rash, breathlessness and hair loss but these only occurred in very small numbers of women and none were significantly different so were not included in the analysis.

Check list for Patients on Endocrine Therapy (C-PET)

To be completed by patient

Name: _____

Date: _____/_____/_____

Hormone treatment for breast cancer sometimes causes side-effects. Please go through this list, and tick (✓) boxes that apply to you, leaving **other boxes blank.** This information will help in your discussions with your nurse or doctor.

To be completed by doctor or nurse

Name: _____

Date: _____/_____/_____

Current treatments: _____

_____

_____

Dose: _____

Use (adjuvant or advance disease)

_____

Symptom	*I am experiencing this symptom*	*I would like to talk to a doctor or nurse about this symptom*	*Comments from medical professionals*
Hot flushes / sweats			
Weight gain			
Nausea			
Low energy			
Fluid retention			
Irritability			
Decreased sexual interest			
Skin rash			
Pain at needle injection site			
Breathlessness			
Vaginal bleeding			
Vaginal discharge			
Vaginal dryness			
Other (please specify)			

Table 1

Side effects results from women taking tamoxifen.

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
1. EF	Y	Y	N	Y	N	N	N
2. HM	N	Y	N	Y	N	N	N
3. SP	N	Y	N	N	N	N	Y
4. 0K	Y	N	N	Y	Y	Y	N
5. CC	N	N	N	N	N	N	N
6. AC	Y	N	N	Y	N	N	N
7. EB	Y	N	Y	Y	N	N	N
8. MC	N	Y	N	Y	Y	N	Y
9. BU	Y	Y	N	Y	Y	N	N
10. EH	Y	Y	Y	N	N	Y	N
11. JB	Y	Y	N	N	Y	Y	Y
12. AS	Y	Y	N	N	Y	N	Y
13. MS	N	N	N	N	N	N	N
14. EB	Y	Y	Y	N	N	N	N
15. JA	Y	Y	Y	Y	Y	N	Y
16.MM	N	N	N	N	N	N	N
17.MW	Y	Y	Y	N	Y	Y	Y
18.MM	N	N	N	N	N	N	N
19. EM	Y	Y	N	Y	N	Y	N
20. MC	N	N	N	N	N	N	Y
21. MS	N	N	N	N	N	N	N
22. CM	N	N	N	Y	N	N	N
23. GK	Y	Y	N	N	N	N	N
24. EB	Y	Y	N	Y	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
25. MG	Y	Y	Y	Y	N	N	Y
26. HD	Y	N	Y	N	N	Y	N
27.MM	Y	N	Y	N	Y	Y	N
28.WK	Y	Y	N	Y	Y	Y	N
29.MK	N	N	Y	N	N	N	N
30.MH	N	Y	N	Y	N	N	Y
31. KR	Y	Y	N	N	N	N	N
32. LR	Y	Y	N	N	N	N	Y
33. ER	N	Y	N	N	N	N	N
34. JR	N	N	Y	N	Y	Y	Y
35. CS	Y	N	N	N	N	Y	Y
36. SR	N	Y	N	N	N	N	Y
37. MT	Y	Y	N	N	Y	Y	Y
38. JU	Y	Y	Y	Y	N	Y	N
39. ED	Y	Y	Y	N	N	N	Y
40. AD	Y	Y	Y	Y	N	Y	N
41.MM	N	N	N	N	N	Y	Y
42. AS	N	N	N	N	N	N	N
43. AM	Y	N	N	N	N	N	N
44. CH	Y	N	N	N	N	N	N
45. SD	N	N	N	Y	N	N	N
46. JA	N	N	N	N	N	N	N
47. GT	N	N	N	N	N	N	N
48. LL	Y	Y	N	N	N	N	N
49. KD	Y	N	Y	N	N	N	Y
50. SM	N	N	Y	N	N	N	Y
51. IN	N	N	N	N	N	N	N
52. NB	Y	Y	N	N	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
53.MM	N	N	N	N	N	N	N
54. DS	Y	Y	N	N	N	Y	N
55. JR	N	N	N	N	N	N	N
56. ES	Y	Y	N	Y	N	N	Y
57. MG	Y	N	N	N	N	N	Y
58. MD	N	Y	N	N	N	N	Y
59. MP	Y	N	Y	Y	N	N	N
60. FB	N	N	N	N	N	Y	Y
61. MR	Y	N	N	N	N	N	Y
62. ES	N	Y	Y	N	N	N	Y
63. CA	Y	Y	N	N	N	N	N
64. PC	Y	N	N	N	N	N	N
65. IH	N	N	N	N	N	N	N
66. FN	N	N	N	N	N	N	N
67. EF	N	Y	Y	N	Y	N	N
68. AL	N	Y	N	Y	N	N	N
69. JC	N	Y	N	Y	N	N	N
70. DM	N	Y	N	Y	N	N	Y
71. FR	N	N	N	N	N	Y	Y
72. JD	Y	Y	N	N	N	N	N
73. JL	N	N	Y	N	N	Y	N
74. IR	Y	N	N	N	N	N	N
75. ED	Y	N	N	N	Y	N	N
76.MD	N	N	N	N	N	N	Y
77. MD	N	N	N	N	N	N	N
78. WP	Y	Y	N	N	N	N	N
79. MT	N	N	Y	N	Y	Y	N
80. DS	N	Y	N	Y	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
81. MH	Y	N	N	N	N	N	N
82. EH	N	N	N	N	N	N	N
83. DR	Y	N	N	Y	N	N	Y
84. ER	N	N	N	N	N	N	Y
85. KM	Y	Y	Y	N	Y	Y	Y
86. JB	Y	Y	Y	N	Y	N	Y
87. DK	Y	N	N	N	N	N	N
88. EG	N	N	N	Y	N	Y	N
89. EC	Y	N	N	N	N	N	N
90. AS	Y	N	Y	N	Y	Y	N
91. BR	Y	Y	Y	N	Y	N	Y
92. GC	N	N	N	N	N	N	Y
93. CR	Y	Y	Y	N	Y	N	N
94. AM	N	Y	N	Y	Y	N	N
95.MM	Y	Y	Y	N	N	N	N
96. JD	Y	Y	Y	Y	Y	N	N
97. AB	Y	Y	N	Y	Y	N	N
98. JD	Y	N	N	N	N	Y	N
99. AC	N	N	N	N	N	Y	Y
100.JB	Y	Y	Y	N	N	N	N
101.JH	Y	Y	Y	N	N	N	N
102.MM	Y	Y	N	N	N	Y	N
103.AG	Y	N	N	N	N	N	N
104.AF	Y	Y	N	N	Y	N	Y
105.IM	Y	Y	Y	Y	N	N	N
106.IM	N	N	N	N	N	N	N
107.RM	Y	N	N	N	N	N	N
108.AD	Y	Y	N	N	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
109.HN	N	Y	Y	N	N	N	N
110.CS	Y	Y	N	Y	N	N	Y
111.AS	Y	N	Y	Y	Y	N	N
112.AS	Y	N	N	N	N	N	N
113.CT	N	N	N	N	N	N	N
114. EW	N	N	N	N	N	N	N
115.WW	Y	Y	Y	Y	Y	N	Y
116.IT	Y	N	N	N	Y	Y	N
117.SC	N	N	N	N	N	N	N
118.EC	N	N	N	N	N	N	Y
119.ML	Y	Y	Y	Y	N	N	Y
120.JM	Y	Y	N	Y	N	N	N
121.EA	Y	N	Y	N	Y	N	N
122.IA	Y	N	Y	Y	N	Y	N
123.AE	Y	Y	N	N	Y	Y	Y
124.DA	Y	N	N	N	N	N	N
125.SA	Y	Y	N	N	Y	Y	Y
126.FA	N	N	N	Y	N	N	Y
127.CA	Y	Y	N	Y	Y	Y	N
128.GA	Y	Y	N	Y	N	N	Y
129.SB	N	N	N	N	N	N	N
130.BB	N	Y	N	N	N	N	N
131.MB	N	Y	Y	N	Y	Y	Y
132.AB	Y	Y	N	N	N	N	N
133.PB	N	N	N	N	N	N	N
134.EB	N	N	N	N	N	Y	N
135.EB	N	N	N	N	Y	N	N
136.EB	Y	N	N	N	N	Y	Y

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
137.KB	N	N	N	Y	N	N	N
138.AB	Y	Y	N	N	N	N	Y
139.MB	N	N	N	N	N	N	Y
140.AB	Y	Y	Y	N	N	N	N
141.DB	Y	N	N	N	N	N	N
142.MB	N	N	Y	N	N	N	Y
143.EB	Y	Y	Y	Y	Y	Y	Y
144.SB	N	N	N	N	N	N	Y
145.HB	Y	N	N	N	N	N	N
146.CB	Y	Y	Y	Y	Y	N	Y
147.MB	Y	Y	N	Y	N	Y	Y
148.AC	N	N	N	N	N	N	N
149.EC	N	Y	N	N	N	N	N
150. SC	Y	N	Y	N	Y	N	Y
151.JC	Y	N	N	N	N	N	N
152.SC	N	Y	N	N	N	N	N
153.IC	N	N	Y	N	N	N	Y
154. PC	Y	Y	N	N	N	N	N
155. LC	Y	Y	N	N	N	N	N
156. BC	Y	Y	Y	N	N	N	N
157. HC	Y	N	N	N	N	N	Y
158. JC	N	N	N	N	N	N	N
159. IC	Y	N	N	N	N	N	N
160. LC	Y	N	N	N	N	N	N
161. NC	N	N	N	N	N	N	Y
162. IC	N	N	N	N	N	Y	Y
163.MC	N	N	Y	N	N	N	N
164. AC	Y	Y	N	N	N	Y	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
165. EC	Y	Y	Y	N	Y	N	N
166.MD	N	N	N	Y	N	N	N
167.VD	N	Y	N	N	N	N	N
168. PD	Y	Y	N	N	Y	N	N
169.MD	Y	Y	N	N	N	N	N
170.ID	Y	Y	N	N	N	N	Y
171.CD	Y	N	Y	N	Y	Y	Y
172.ED	Y	Y	Y	N	N	N	Y
173.GD	Y	N	N	Y	N	N	N
174.CD	N	N	N	N	Y	Y	N
175.MD	Y	Y	N	N	N	Y	Y
176.PD	N	N	N	N	N	N	N
177.RE	N	Y	N	N	N	N	N
178.EE	Y	N	N	N	Y	Y	Y
179.FF	Y	Y	Y	Y	N	N	Y
180.IF	N	N	Y	N	N	N	N
181.JF	N	N	N	N	Y	Y	Y
182.MF	Y	Y	N	N	Y	N	Y
183.EF	N	N	Y	N	N	Y	N
184.MF	Y	N	N	N	N	N	N
185.SF	Y	N	N	N	N	N	N
186.TF	Y	Y	Y	Y	Y	Y	N
187.SF	Y	N	Y	N	N	N	Y
188.MF	N	Y	N	Y	N	N	N
189.MF	N	N	N	N	N	N	N
190.JF	Y	Y	Y	N	N	N	N
191.HF	Y	N	Y	N	Y	N	Y
192.NG	Y	Y	N	Y	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
193.MG	Y	Y	N	N	Y	Y	N
194.DG	N	N	N	N	N	N	N
195.SG	N	N	N	N	N	N	Y
196.JG	Y	N	N	Y	Y	N	N
197.EG	N	Y	N	Y	N	Y	N
198.SG	Y	N	N	N	N	N	N
199.EH	Y	N	N	N	N	N	Y
200.MH	Y	N	N	N	Y	N	Y
201.PH	Y	Y	Y	N	Y	Y	N
202.MH	N	N	N	N	N	N	Y
203.AH	Y	N	Y	N	N	N	N
204.CH	Y	N	N	N	N	N	Y
205.MH	Y	N	N	N	N	N	N
206.MH	N	N	N	N	N	N	N
207.EH	Y	N	N	Y	N	N	N
208.CH	Y	N	Y	N	N	N	Y
209.CH	N	N	N	N	N	N	N
210.DH	Y	Y	N	Y	Y	N	Y
211.AI	Y	Y	Y	Y	N	Y	N
212.SI	Y	N	N	Y	N	Y	N
213.JJ	Y	Y	Y	Y	N	N	N
214.MJ	Y	Y	Y	N	Y	N	N
215.CJ	Y	Y	Y	Y	Y	Y	Y
216.SJ	N	Y	N	N	N	N	Y
217.MJ	Y	N	N	N	N	Y	Y
218.NJ	Y	N	N	N	N	N	Y
219.AJ	Y	N	N	N	N	N	Y
220.MJ	Y	Y	N	N	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
221.MK	Y	Y	Y	Y	Y	Y	Y
222.JK	Y	N	N	N	N	Y	Y
223.PK	Y	Y	N	N	N	Y	N
224.AK	N	N	N	N	N	N	N
225.GK	N	Y	N	Y	N	Y	Y
226.TK	N	Y	N	N	N	Y	N
227.WL	Y	N	N	N	Y	N	N
228.EL	Y	N	N	N	N	N	N
229.AL	N	N	N	N	N	N	N
230.DL	N	N	N	N	N	N	N
231.ML	Y	N	N	N	N	N	N
232.PL	Y	Y	N	N	N	Y	Y
233.AL	Y	N	N	N	Y	Y	N
234.GL	Y	Y	N	N	Y	N	N
235.JL	N	N	N	N	N	N	Y
236.RM	N	N	N	Y	N	N	Y
237.MM	N	N	N	Y	N	N	N
238.EM	N	N	N	N	N	N	Y
239.SM	Y	Y	N	Y	Y	N	Y
240.MM	N	N	N	N	N	N	N
241.EM	N	N	Y	N	N	N	Y
242.IM	Y	N	N	N	N	N	Y
243.AM	N	Y	Y	N	N	Y	Y
244.CM	Y	N	N	N	N	N	N
245.IM	N	Y	Y	Y	N	N	Y
246.GM	N	Y	N	Y	N	Y	Y
247.AM	N	N	N	N	N	N	N
248.NM	Y	Y	N	Y	N	N	Y

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
249.AM	N	N	N	N	N	N	N
250.IM	Y	N	N	N	N	N	N
251.SM	Y	Y	Y	N	Y	N	Y
252.JM	N	Y	N	N	N	N	N
253.JM	Y	N	N	N	N	N	N
254.SM	Y	N	N	N	N	N	N
255.JM	N	N	Y	Y	Y	Y	N
256.JM	Y	N	N	Y	N	N	Y
257.MM	N	N	N	N	N	N	Y
258.IM	Y	N	N	N	N	N	Y
259.HM	Y	N	N	N	Y	Y	N
260.OM	Y	N	N	N	N	N	N
261.MM	Y	Y	Y	Y	N	Y	Y
262.AM	N	N	N	N	N	N	N
263.BN	Y	Y	N	Y	N	N	N
264.MN	Y	Y	Y	Y	N	N	Y
265.EN	N	N	N	N	N	N	Y
266.AN	Y	N	N	N	N	N	N
267.FO	Y	Y	N	N	Y	N	Y
268.EO	Y	Y	N	Y	N	N	N
269.JP	N	N	N	N	N	N	Y
270.CP	N	Y	Y	N	N	N	Y
271.KP	N	Y	N	N	N	N	N
272.EP	Y	Y	N	Y	Y	Y	Y
273.MP	Y	N	N	N	Y	N	N
274.MP	N	N	Y	Y	N	Y	Y
275.JP	N	Y	N	N	N	N	Y
276.MR	N	N	N	N	N	N	Y

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
277.GR	Y	N	N	N	N	N	N
278.LR	Y	Y	Y	N	Y	N	Y
279.JR	Y	Y	Y	N	Y	Y	Y
280.AR	Y	N	N	N	N	Y	N
281.MR	Y	Y	N	Y	N	N	Y
282.JR	N	N	N	N	N	N	N
283.IR	N	N	Y	N	N	Y	N
284.MR	N	Y	N	N	N	N	Y
285.HR	Y	Y	N	Y	Y	N	Y
286.JR	Y	Y	N	N	N	N	Y
287.ER	Y	N	N	N	N	N	Y
288.CR	Y	N	N	N	Y	N	Y
289.RS	N	N	Y	N	Y	Y	Y
290.MS	N	N	N	N	N	N	N
291.AS	Y	Y	N	N	N	N	Y
292.TS	Y	Y	N	Y	N	Y	N
293.ES	Y	Y	Y	Y	N	N	Y
294.SS	N	N	N	N	N	N	Y
295.TS	N	Y	Y	N	N	N	N
296.KS	N	N	N	N	N	N	Y
297.MS	Y	N	N	N	N	N	Y
298.RS	Y	N	N	N	N	N	N
299.GS	Y	N	Y	Y	Y	Y	Y
300.CS	N	Y	N	N	N	N	N
301.MS	Y	Y	N	N	N	Y	N
302.AS	Y	N	N	N	Y	N	N
303.RS	Y	Y	N	N	N	N	N
304.AS	N	Y	N	N	N	N	Y

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
305.JS	Y	N	N	N	N	N	N
306.KS	Y	Y	N	N	Y	Y	Y
307.CS	Y	N	N	N	N	N	N
308.SS	N	N	N	N	N	N	Y
309.RS	Y	N	Y	N	N	N	N
310.MS	N	N	N	N	N	N	N
311.AS	N	N	N	N	N	N	N
312.AS	Y	N	N	N	N	Y	Y
313.IS	N	N	N	N	N	N	N
314.ES	Y	Y	N	N	Y	N	Y
315.NS	Y	N	N	N	Y	Y	N
316.CS	Y	Y	N	N	N	N	N
317.AT	N	Y	N	N	N	N	N
318.MT	N	N	N	N	N	N	N
319.RT	Y	N	N	N	N	N	N
320.CT	N	N	N	N	N	N	Y
321.CT	Y	N	N	N	N	Y	N
322.HW	Y	N	N	N	N	N	Y
323.MW	N	N	Y	N	N	N	N
324.LW	Y	N	N	N	N	Y	N
325.MW	Y	Y	N	Y	Y	N	Y
326.YW	Y	Y	Y	Y	N	Y	Y
327.WW	Y	Y	N	Y	N	N	N
328.JW	Y	Y	N	N	Y	Y	Y
329.JW	N	N	N	N	N	N	N
330.VW	N	N	N	N	N	N	N
331.GY	N	N	N	Y	N	N	N
332.AD	Y	N	N	N	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
333.MG	Y	Y	N	N	N	Y	Y
334.CS	Y	Y	Y	Y	N	N	Y
335.AS	N	N	N	N	N	N	N
336.IW	N	Y	N	N	N	N	N
337.JT	N	N	N	N	N	N	Y
338.EH	N	N	N	N	N	N	Y
339.MR	Y	N	N	N	N	N	N
340.EC	N	Y	N	N	N	N	Y
341.MD	Y	N	N	N	N	N	N
342.AM	N	Y	N	N	N	N	Y
343.MM	Y	N	N	N	N	N	Y
344.MC	Y	N	N	N	N	N	Y
345.MD	Y	Y	N	N	N	N	Y
346.YH	N	N	N	N	N	N	N
347.HS	Y	Y	N	Y	N	N	Y
348.JP	Y	Y	Y	Y	N	N	N
349.ED	N	N	Y	N	N	N	N
350.JF	N	N	N	N	N	N	Y
351.IW	Y	Y	N	Y	N	N	N
352.JH	N	N	N	N	N	Y	Y
353.NT	Y	N	Y	N	Y	Y	Y
354.ES	Y	N	N	N	N	N	N
355.JK	N	N	N	N	N	N	Y
356.RB	N	Y	Y	Y	Y	Y	Y
357.MM	Y	Y	Y	N	Y	N	Y

Table 2

Side effects results from control patients.

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
1. RC	Y	N	N	N	N	N	N
2. WP	N	N	N	N	N	N	N
3. IW	Y	Y	N	N	Y	Y	N
4. AH	N	Y	N	N	N	N	N
5. FS	N	N	N	N	N	Y	N
6. SM	N	N	Y	N	N	N	Y
7. PM	N	N	Y	N	N	N	N
8. AJ	Y	Y	Y	N	N	Y	N
9. MM	N	Y	N	Y	N	Y	N
10. IW	N	N	N	N	N	N	N
11. MG	N	Y	Y	Y	N	Y	Y
12. MM	Y	N	Y	Y	N	N	N
13. JR	N	N	N	N	N	Y	N
14. ES	Y	Y	N	N	N	Y	N
15. ME	N	Y	N	N	N	N	N
16. SJ	N	N	N	N	N	N	N
17. IG	N	N	N	N	N	N	N
18. CL	N	Y	Y	N	Y	N	N
19. EM	Y	N	Y	N	N	N	Y
20. EH	Y	N	Y	N	N	N	N
21. RB	Y	Y	Y	Y	Y	N	N
22. CH	Y	N	N	N	N	N	N
23. CR	N	N	N	N	Y	N	N
24. KT	Y	N	N	N	N	N	Y

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
25. EM	N	N	N	N	N	N	Y
26. AB	N	N	Y	N	Y	Y	N
27. MA	Y	Y	N	N	Y	Y	Y
28. EA	N	N	N	N	N	N	N
29. MM	N	Y	N	N	N	N	N
30. AN	N	Y	N	N	N	Y	N
31. BA	Y	N	N	N	N	N	N
32, RW	N	N	N	N	N	N	N
33. ER	Y	N	N	N	N	N	N
34. MM	N	N	N	N	N	N	N
35. BL	Y	Y	N	N	N	N	N
36. ER	N	Y	N	N	N	Y	N
37. JW	Y	N	N	N	Y	N	N
38. MM	Y	N	Y	N	N	N	N
39. AC	Y	N	N	N	Y	Y	N
40. BM	N	Y	N	N	N	N	N
41. CP	N	Y	Y	N	N	N	Y
42. MC	Y	N	N	N	N	N	N
43. MM	N	N	N	N	N	Y	N
44. MD	Y	Y	N	Y	N	N	N
45. MB	Y	Y	Y	N	Y	Y	N
46. EM	Y	N	N	N	N	N	N
47. IR	Y	Y	N	Y	N	N	N
48. LG	N	N	N	N	N	N	N
49. RK	N	Y	Y	N	N	N	N
50. AB	Y	N	N	N	N	N	N
51. DB	N	N	N	N	Y	Y	Y
52. AG	N	N	N	N	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
53. MB	N	Y	N	N	N	N	N
54. AB	Y	N	N	Y	Y	Y	N
55. LA	N	N	N	N	N	Y	N
56. IC	N	N	Y	N	N	N	N
57. AB	Y	Y	Y	N	N	N	N
58. MP	Y	Y	Y	N	N	N	N
59. MC	Y	N	N	N	Y	Y	N
60. JS	N	Y	N	N	N	N	N
61. ES	N	N	N	N	N	N	N
62. HM	Y	Y	Y	N	N	N	N
63. MP	N	N	Y	N	Y	N	N
64. MM	Y	Y	N	N	Y	Y	N
65. SP	N	N	Y	N	N	N	N
66. MB	Y	N	N	Y	Y	Y	N
67. MM	N	N	N	N	N	N	N
68. MA	N	N	N	N	N	N	N
69. SB	N	N	N	N	N	N	N
70. CM	N	N	N	N	N	N	N
71. AH	N	N	N	N	N	N	N
72. IW	Y	Y	N	N	N	N	N
73. MW	N	N	N	Y	N	N	N
74. IG	N	N	N	N	N	N	N
75. AW	N	N	N	N	Y	Y	N
76. AS	Y	N	Y	N	Y	Y	Y
77. JM	N	N	N	N	N	N	N
78. JD	N	N	N	Y	N	Y	N
79. BW	N	N	N	N	N	N	N
80. AS	Y	Y	Y	N	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
81. MA	Y	Y	Y	N	N	N	N
82. EG	Y	N	N	N	Y	N	Y
83. SM	N	Y	N	Y	N	N	N
84. MM	Y	Y	N	N	N	N	N
85. MM	N	N	N	N	N	N	N
86. MF	N	N	N	N	N	N	N
87. MA	Y	Y	N	N	N	N	N
88. AH	N	Y	N	N	N	N	N
89. MG	N	Y	N	N	N	Y	N
90. PT	N	N	N	N	N	N	N
91. DM	N	N	N	N	N	N	N
92. AS	N	Y	N	N	N	N	N
93. AS	Y	Y	Y	N	Y	N	N
94. JC	Y	N	N	N	N	Y	N
95. IV	Y	N	N	N	N	N	N
96. KL	N	N	N	N	N	N	N
97.	N	N	N	N	N	N	N
98. MG	Y	Y	N	N	N	N	N
99. MP	N	N	N	N	N	Y	N
100. PM	N	N	N	N	N	N	N
101.MM	Y	N	N	N	N	Y	Y
102.EM	Y	N	N	N	N	N	N
103.NL	Y	N	N	N	N	N	N
104. PL	N	N	Y	N	Y	N	Y
105.EB	Y	N	N	N	N	Y	N
106.BM	Y	N	N	N	N	N	N
107.MB	N	Y	Y	N	N	N	N
108.MM	N	Y	Y	N	N	N	N

Patient	hot flushes sweats	weight gain	low energy	fluid retention	low libido	vaginal dryness	vaginal discharge
109.BW	Y	N	N	N	N	N	N
110.AM	N	Y	N	N	N	N	Y
111.ES	N	Y	N	N	Y	N	N
112. JR	N	N	Y	N	Y	N	N
113. CR	Y	Y	N	N	N	N	N
114. IO	Y	Y	Y	Y	Y	N	N
115.EM	Y	Y	Y	Y	Y	N	N
116. PC	N	Y	N	Y	N	N	N
117.HA	Y	N	N	N	Y	Y	Y
118.MW	N	N	N	N	N	N	N
119.WB	N	N	N	N	N	N	N
120.SM	Y	N	Y	N	Y	Y	N
121.MS	Y	N	N	N	N	N	N
122.MR	Y	N	N	N	N	Y	Y
123.EC	Y	N	N	N	N	N	N
124.MD	Y	Y	Y	Y	N	Y	N
125.DW	Y	Y	N	N	N	Y	N
126.JL	Y	N	Y	N	Y	N	N
127.SN	Y	N	Y	N	Y	N	N
128.MS	N	N	Y	N	N	N	N
129.NM	N	N	N	N	N	N	N
130.JK	N	N	Y	N	N	N	Y

Figure 1
Tamoxifen side effects versus control group.

Discussion

Tamoxifen is the most common chemotherapeutic agent in current use. Studies have shown it to be of benefit in pre and postmenopausal patients with oestrogen receptor positive breast cancer in terms of up to 40% decrease in local recurrence, distal metastases and contralateral cancer. It has been used to treat over 3 million women worldwide. However, no drug is without side effects and tamoxifen has a number of recognised toxic and non-toxic effects.

Some of the toxic effects of tamoxifen reported include ocular toxicity, liver derangement, haematological changes (leucopenia, thrombocytopaenia), hypercalcaemia, tamoxifen flare and its effect on the endometrium.

Toxic effects requiring discontinuation of the drug are rare and withdrawal from treatment occurs in less than 3% of patients overall [Heel 1978]. The Christie trial reported that 2% of women had to reduce the dose of tamoxifen to 10mg/day because of distressing side effects and 4% stopped the drug in less than 1 year [Riveiro 1988]. In the Scottish trial, 28 of the 641 patients given adjuvant tamoxifen stopped the drug because of toxicity (4%), mostly during the first year of therapy [Scottish 1987]. Both trials refer to tamoxifen being discontinued because of toxicity but neither specifies what side effects were experienced, ie whether they were distressing but not life-threatening, eg hot flushes/sweats or more serious, eg ocular, hepatic or haematological effects.

An association between tamoxifen and ocular disease was first recognised in 1978 and various effects have been reported including retinopathy, optic neuritis, corneal changes, macular oedema and keratopathy. Kaiser-Kupfer [1978] reported 4 cases of ocular toxicity amongst a group of women receiving high dose tamoxifen (120-320 mg/day) for metastatic breast cancer. These patients who had taken tamoxifen for 17-27 months developed a loss in visual acuity as a result of retinopathy affecting mainly the macula and accompanied by macular oedema. 3 of the 4 patients also had unusual corneal changes [Vizel 1982].

Approximately 21 cases of tamoxifen related ocular toxicity have been reported as case reports and the most common clinical abnormality is decreased central visual acuity which

has been reported in 16 of the 21 cases. 10 of these 16 patients experienced improvement in their vision following cessation of tamoxifen [Nayfield 1996]. Both development of ocular toxicity and potential improvement in visual acuity may be related to total cumulative dose of tamoxifen. Most of the reports have been associated with high dose tamoxifen or high cumulated doses although there have been a few reports at conventional dosage [McKeown 1981, Kaiser-Kupfer 1978]. Longstaff et al [1990] found no ocular toxicity in a blind study comparing standard dose tamoxifen to controls.

Overall, therefore, the incidence of any ocular complications from tamoxifen is low. However, if caught early, any complications may prove reversible. Although there is little evidence to support regular opthalmological screening of women on tamoxifen, anyone presenting with visual disturbances or a worsening of visual acuity should be investigated [Nayfield 1996].

Thromboembolic complications have also been reported with tamoxifen therapy [Hendrick 1980]. Lipton et al [1984] reported 7 patients who developed venous thrombosis or pulmonary embolism within 6 months of starting tamoxifen treatment. This was in a group of 220 patients, giving an incidence of 3.2%. Jungi, Wagenknecht and Lerner have also reported pulmonary emboli in patients during tamoxifen administration [Heel 1978]. However, the mechanism for this is not clear and a causal relationship has not been established. Some studies suggest this may be due to a decrease in functional activity of antithrombin III [Enck 1984, Lipton 1984, Love 1989] but studies from Jordan, Auger and Mackie do not support this theory. Evidence from the large tamoxifen trials suggest a small increased incidence of thrombophlebitis only [Enck 1984, Lipton 1984, Love 1989].

Hypercalcaemia may be an early complication of tamoxifen therapy. In a study by Legha et al [1981] 2.3% of patients with metastatic breast cancer developed hypercalcaemia within a median of 7 days (range 4-11) of starting tamoxifen therapy. All patients had bone metastases. Hypercalcaemia is a well known complication of hormonal therapy in breast cancer using oestrogens and androgens. Hormone induced hypercalcaemia usually has a rapid onset (within 5-10 days of starting hormonal therapy) and calcium levels return to normal when the drug is stopped. It can occur spontaneously in 10-25% of breast cancer patients especially when osteolytic bone metastases are present, but tends to occur gradually

and therefore the symptoms develop slowly. Some have suggested that hypercalcaemia along with 'tamoxifen flare' may signify tumour response [Villalon 1979, Arnold 1979]. Although tamoxifen is an antioestrogen, its apparent hypercalcaemic effects appear to be similar to oestrogen induced hypercalcaemia therefore this may represent one of tamoxifen's partial oestrogenic effects [O'Connell 1981]. Legha [1981] concluded that although hypercalcaemia occurs infrequently, if patients have proven bone metastases then calcium levels should be checked regularly during the first few weeks of tamoxifen therapy and that tamoxifen should be continued or interrupted only until calcium levels fall again. This side effect is potentially serious but is usually short lived and requires supportive measures only. Although tamoxifen was developed as an antioestrogen it has been found to have partial oestrogen actions, of which hypercalcaemia may be one.

Hepatotoxicity is another partial oestrogenic side effect. However, the concern about hepatocellular carcinoma is based on laboratory studies alone [Jordan 1995]. There have been several studies of large doses of tamoxifen inducing liver tumours in rats but this has never been reported in humans at a standard 20mg/day dose. There have been 2 reports of women developing hepatocellular carcinoma who took 40mg/day of tamoxifen but in a tumour that is very rare in the general population anyway, cause and effect is difficult to prove.

Another important partial oestrogenic side effect of tamoxifen is its effect on the endometrium and its use is associated with an increased incidence of both benign and malignant endometrial lesions. The incidence of endometrial cancer is increased in breast cancer patients on tamoxifen from 1:1000 women/year to 2:1000 women/year [Jaiyesimi 1995]. The endometrial effect of tamoxifen is addressed in detail in the earlier ***Pilot Study and Endometrial Screening sections.***

However not all of tamoxifen's oestrogenic effects are detrimental. Tamoxifen is known to act as a partial oestrogen on bone and the cardiovascular system which may prove of some benefit [Love 1992, 1994, Jordan 1987, 1999, McDonald 1995]. Recent studies have suggested a minimal increase in bone mineral density in women taking tamoxifen, which, particularly in postmenopausal women confers a protective effect similar to that seen with hormone replacement therapy. This may reduce the fracture rate in postmenopausal

women, which causes considerable morbidity and mortality in this group. Love et al [1992] found that tamoxifen use was associated with a significant preservation of the bone mineral density of the lumbar spine in postmenopausal women but were unable to determine whether this would correlate with a decrease in the risk of fractures. Turken et al [1989] found similar but non-significant results. Jordan et al [1987] studied the effect in rats and suggested that tamoxifen helped to maintain bone density such that tamoxifen may be useful in retarding the development of osteoporosis and thus, logically, fractures as a result of that.

Tamoxifen also causes oestrogenic changes in blood lipids by reducing low density lipoproteins and total cholesterol and increasing high density lipoproteins. This may have the effect of reducing cardiovascular disease which in postmenopausal women remains the biggest cause of morbidity and mortality. Powles et al [1990] in their prevention study reported that tamoxifen significantly reduced serm cholesterol, fasting low density lipoproteins and apoprotein B levels in postmenopausal women. In premenopausal women the effect on serum cholesterol and LDL was present but smaller and there was no significant effect on other lipid and lipoprotein fractions. The Scottish trial reported a reduction in the incidence of fatal myocardial infarction in postmenopausal women on tamoxifen as compared to controls and the Stockholm trial showed a reduction in the incidence of hospital admissions for cardiovascular illness in tamoxifen users as compared to controls. The NATO trial also reported a reduction in non cancer deaths in tamoxifen users [NATO 1988, Jaiyesimi 1995].

The vast majority of women who receive tamoxifen will never experience any toxic side effects. However, a number of other non life-threatening effects have been reported commonly including hot flushes/sweats, weight gain, fluid retention, nausea and vomiting, reduced libido, vaginal dryness, vaginal discharge, skin rash and non specific central nervous system symptoms including depression, irritability, headache, sleep disturbance and lethargy. Depression has been reported in 1-15% of women taking tamoxifen and been specifically attributed to tamoxifen [Shariff 1995]. Although non toxic, these side effects can be extremely distressing as they affect daily living and quality of life long term. The most distressing and frequent effect is hot flushes/sweats and in the population studied there was a significant difference between the study population and controls ($p=0.0099$). Other

studies have reported a similar significant difference [Love 1991, Ray 1996]. Some women report a reduction in vasmotor symptoms over time and there are various treatments available, eg clonidine, venlafaxine and megestrol acetate which have varying success, but despite this it still remains a very distressing symptom [Love 1993, Loprinzi 1994].

A number of women report weight gain in association with tamoxifen use. Although there was a difference between the study group and controls with regard to weight gain it was not statistically significant and may reflect a general decrease in self-esteem and negative body image following breast surgery. Kumar et al [1997] looked at weight gain associated with tamoxifen therapy and also found that although there was a mean weight gain of 1.2kg in all patients, there was no significant difference for those receiving tamoxifen against those not receiving tamoxifen and weight gain during treatment with tamoxifen was not correlated with treatment duration.

Another highly significant side effect found in the population studied was vaginal discharge ($p<0.0001$). Ray et al [1996] also reported a significant difference in vaginal discharge and Love et al [1993] reported an increase in gynaecological symptoms (unspecified). Only 5% (a non significant difference) reported vaginal discharge in a study by Powles et al [1989] to evaluate the acute toxicity and feasibility of tamoxifen for the prevention of breast cancer. Sobel [1996] reported specifically on an increase in vulvovaginal candidiasis in postmenopausal women secondary to tamoxifen. The present study did not assess whether discharge was secondary to infection. This effect almost certainly reflects the partial oestrogenic nature of tamoxifen on the genital tract. Boccardo [1981] and Ferrazzi [1997] found an increase in the karyopicnotic index of vaginal tissue of women on tamoxifen reflecting oestrogenisation of the vaginal tissue similar to that of premenopausal women. Patients can be reassured that unless the discharge is blood stained/brown it is not significant. Many postmenopausal women expressed concern about whether the discharge experienced was normal or not but when reassured did not report that they found this effect distressing.

Fluid retention was also reported significantly more often in women taking tamoxifen than in the control population. This could also be a partial oestrogenic effect of tamoxifen in that in premenopausal women fluid retention is experienced most commonly

premenstrually. Oedema has been reported in 2-7% of patients in studies by Cole, Ferrazzi and Lerner [Heel 1978] and in combination with a degree of weight gain may prove a very distressing symptom.

Thus, although these symptoms are not life threatening they occur in a significant number of women taking tamoxifen who find them distressing and which affect quality of life. They may in some instances cause women to decrease the dose of tamoxifen taken or even stop tamoxifen altogether. Women should be warned about the possible side effects, toxic and non-toxic, and be adequately supported whilst taking the drug.

Anecdotally women report differences in severity of some side effects, eg hot flushes and nausea with tamoxifen produced by different pharmaceutical companies and it may be worth trying a different preparation of the drug if symptoms prove troublesome.

There are several options for the treatment of common side effects, in particular hot flushes. Megestrol acetate (megace), a progestogen, can be effective as can other centraly acting agents such as venlafaxine (a serotonin and noradrenaline reuptake inhibitor) or prozac (fluoxetine). For vaginal dryness, replens, a non hormonal vaginal preparation which has an acid pH, can be tried initially but if proves inadequte, topical oestrogen preparations such as vagifem (which has minimal systemic uptake) are more effective.

TAMOXIFEN METABOLITES

Introduction

[trans-1-(4-[2-dimethylamino)ethoxy]phenyl-1,2-diphenylbut-1-ene] - Tamoxifen, is readily absorbed following oral administration, has a long plasma half-life of 7 days and reaches steady state levels by the end of the first month of treatment [Jordan 1990]. Serum levels of tamoxifen at steady states are extremely variable (50-300ng/ml) and blood levels of tamoxifen have been shown not to correlate with breast cancer response [Etienne 1989, Jaiyesimi 1995, Lerner 1990]. There are, however, no data relating serum tamoxifen levels to endometrial abnormalities.

The aim was to establish the relationship between serum tamoxifen levels and endometrial abnormalities to determine whether increased plasma levels correlated with increased incidence of endometrial abnormalities. All women are prescribed 20mg/day tamoxifen irrespective of height and weight (body mass index [BMI]). Chemotherapeutic agents are adjusted for the above factors but traditionally all hormonal agents have been prescribed irrespective of BMI. One theory is that high plasma levels of tamoxifen might correlate with the presence of endometrial abnormalities. However, the very low incidence of abnormalities detected in this present study group was such that it was the aim to study the relationship between plasma tamoxifen levels and endometrial thickness on ultrasound.

Tamoxifen is extensively metabolised in patients to primarily 4-hydroxytamoxifen and desmethyltamoxifen. Desmethyltamoxifen accounts for about 50-75% of metabolites but is actually a relatively weak antioestrogen. A further 25% remains as tamoxifen itself and the remainder consists of 4-hydroxytamoxifen and other metabolites. Although 4-hydroxytamoxifen is present at very low concentrations in serum it has an extremely high binding affinity for the oestrogen receptor and therefore plays a significant role in the antitumour activity of tamoxifen. There is, however, a large inter-patient variability in 4-hydroxytamoxifen levels [Etienne 1989].

Metabolites Z, Y(from N-desmethyltamoxifen), E and bisphenol have also been discovered; metabolite E and bisphenol are oestrogenic metabolites as they both lack the

dimethylaminoxy side chain necessary for antioestrogenic properties [Osborne 1994].

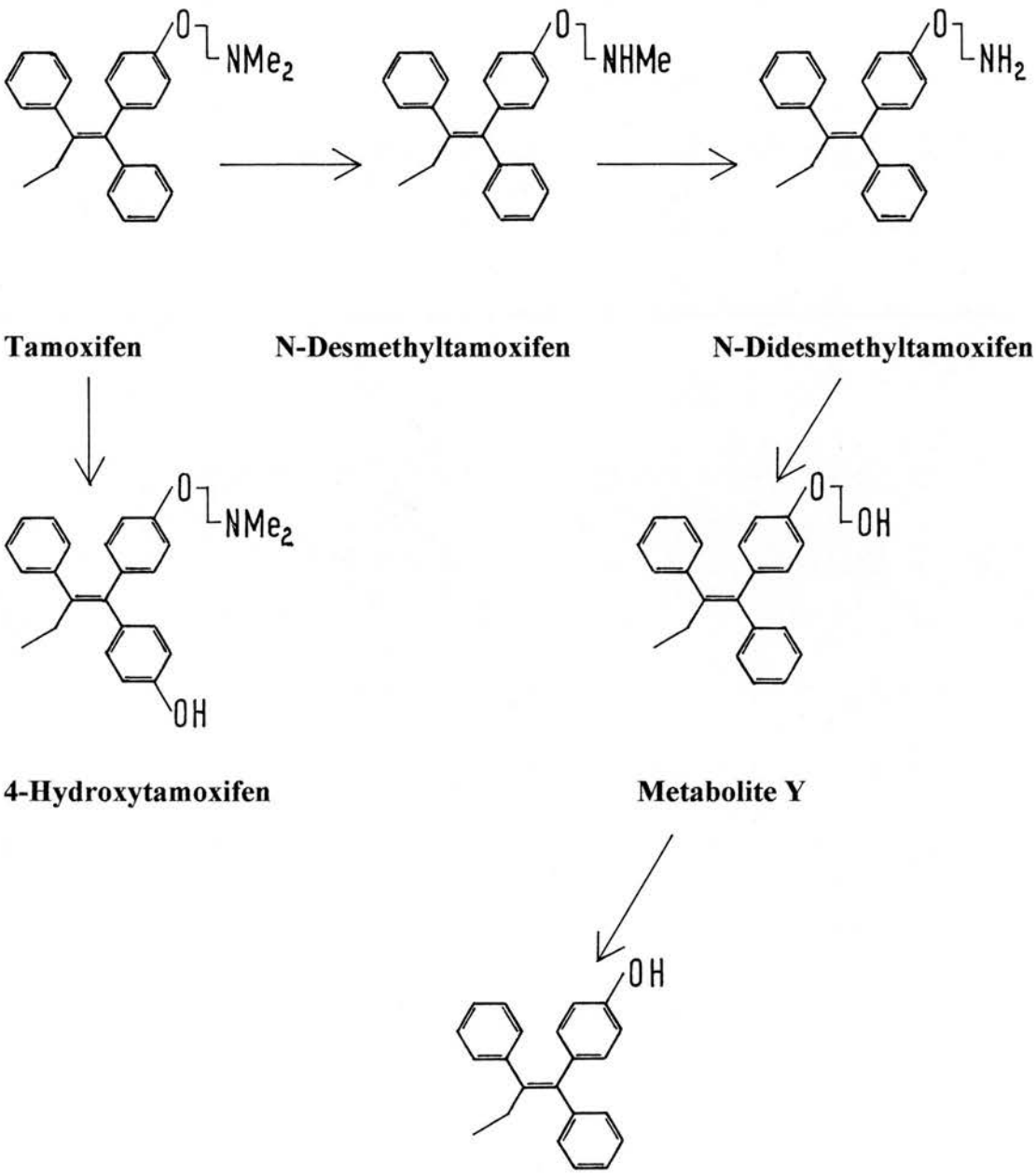
Figure 1 shows tamoxifen and its main metabolites [Lerner 1990, Poon 1993].

A review of the literature indicated that not only were there no data comparing plasma tamoxifen levels with endometrial abnormalities but that the 20mg/day dose of tamoxifen that is most commonly prescribed appeared to have been arrived at empirically and indeed doses of 40mg or 60mg daily have been prescribed in some countries. All women receive the same dose irrespective of BMI and although there is evidence that blood levels of tamoxifen do not correlate with breast cancer response, there is a wide variation in steady state plasma levels and there is no explanation for this. A secondary aim was to establish whether BMI influenced plasma levels and could be an explanation for the wide variation in steady state plasma levels.

The analysis of tamoxifen and its metabolites is difficult because of low plasma concentrations and light instability of the target compounds [Fried 1994]. High performance liquid chromatography is used initially and then sensitivity enhanced by photochemical activation which converts tamoxifen by UV radiation, from a phenylethylene to a phenanthrene. Levels are estimated following UV fluorescence. Thin layer chromatography has also been used as an alternative to HPLC but these methods are time consuming and result in variable extraction efficiencies (60-90%). A new method was established in Edinburgh which included a rapid and simple solid phase extraction making it feasible to assess plasma levels for the whole study group [MacCallum 1996].

Figure 1

Tamoxifen and its major metabolites.



Tamoxifen **N-Desmethyltamoxifen** **N-Didesmethyltamoxifen**

4-Hydroxytamoxifen **Metabolite Y**

Metabolite E

Patients and Methods

Although samples were taken from all 357 women to measure plasma tamoxifen and its metabolites 4-hydroxytamoxifen and desmthyltamoxifen results were obtained for only 160 women. Whilst all 357 samples were prepared for analysis, as described previously in ***Methods: Tamoxifen Metabolite Plasma Levels*** there were problems with the HPLC which led to reduced extraction rates. Extraction rates of <50% were not analysed. During the method development extraction efficiencies of approximately 60% were achieved and between day variation in extraction efficiencies was low (<10%). For the purposes of this study extraction efficiencies were calculated for each sample run from cis spiked plasma compared to cis/MeOH standard and if the efficiency of each run was less than 50% then the sample result was discarded.

During the course of the study there were separate problems with the autosampler and the beam boost which led to a time delay between sample extraction and HPLC. The prolonged storage of samples may have affected the sample and thus the extraction rates. However, the technique used had been previously validated by storing patients plasma samples at -40°C and assaying these stored samples at regular intervals (1, 2, 4, 8 and 12 months). These showed no detectable degradation and therefore this was not thought to be the main cause of difficulties. Time constraints did not allow rerun of the samples with reduced extraction rates so the following results pertain to samples with extraction rates >50%.

During the method development tamoxifen (TAM), 4-hydroxytamoxifen (4OHT), desmethyltamoxifen (DMT) and cis-tamoxifen were measured but cis-tamoxifen was detectable in only 3/59 (5%) of plasma samples so was not measured as part of this study. Standard curves were constructed for each metabolite (Figure 2) which were prepared using 7 replicate concentrations (over a range of 0.002 - 2μg/ml) of mixed metabolites in MeOH and plasma. Mean values were plotted as concentration versus area under the curve which was the area taken from the plot produced by the integrator for each sample. Standard curve equations for the line of best fit were then used for the calculation of metabolite concentration in the study samples.

From each sample a trace was obtained from the integrator (Figure 3).

Figure 2

Tamoxifen standard curve.

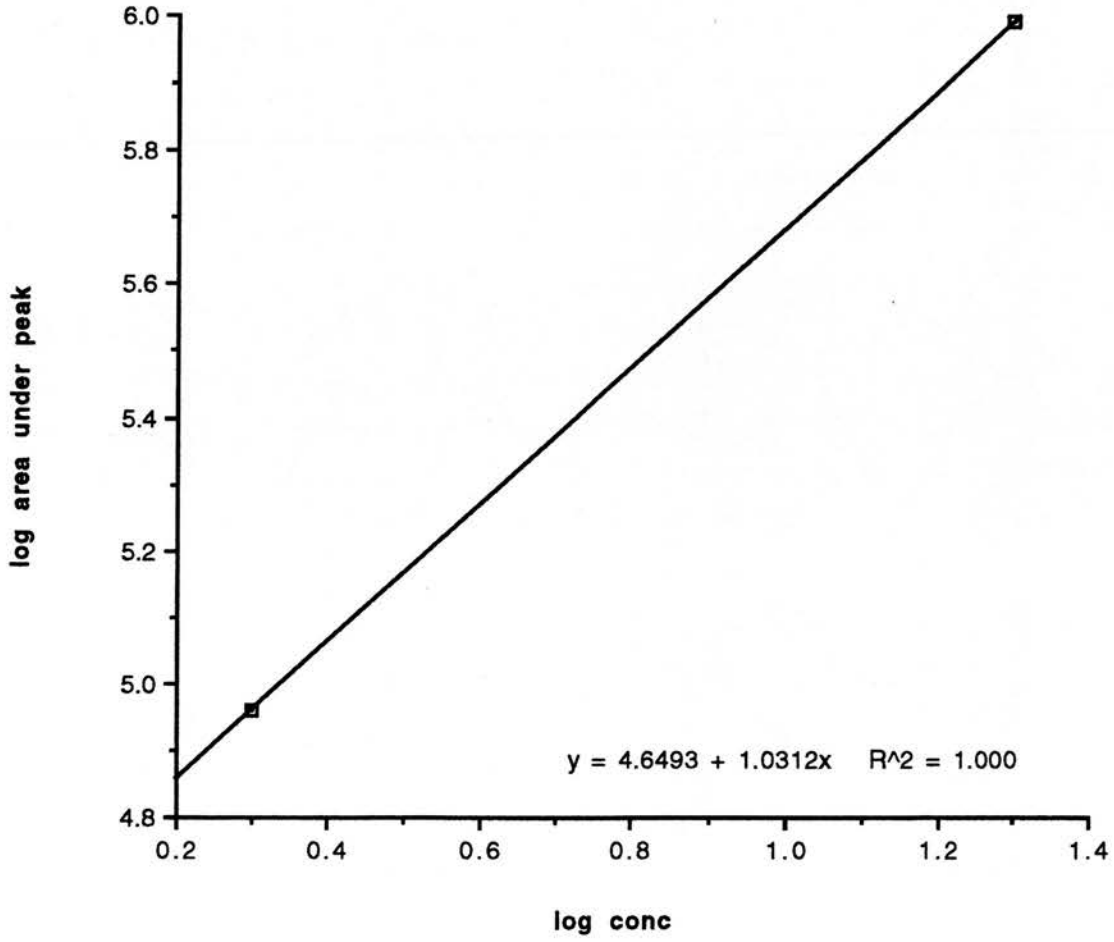
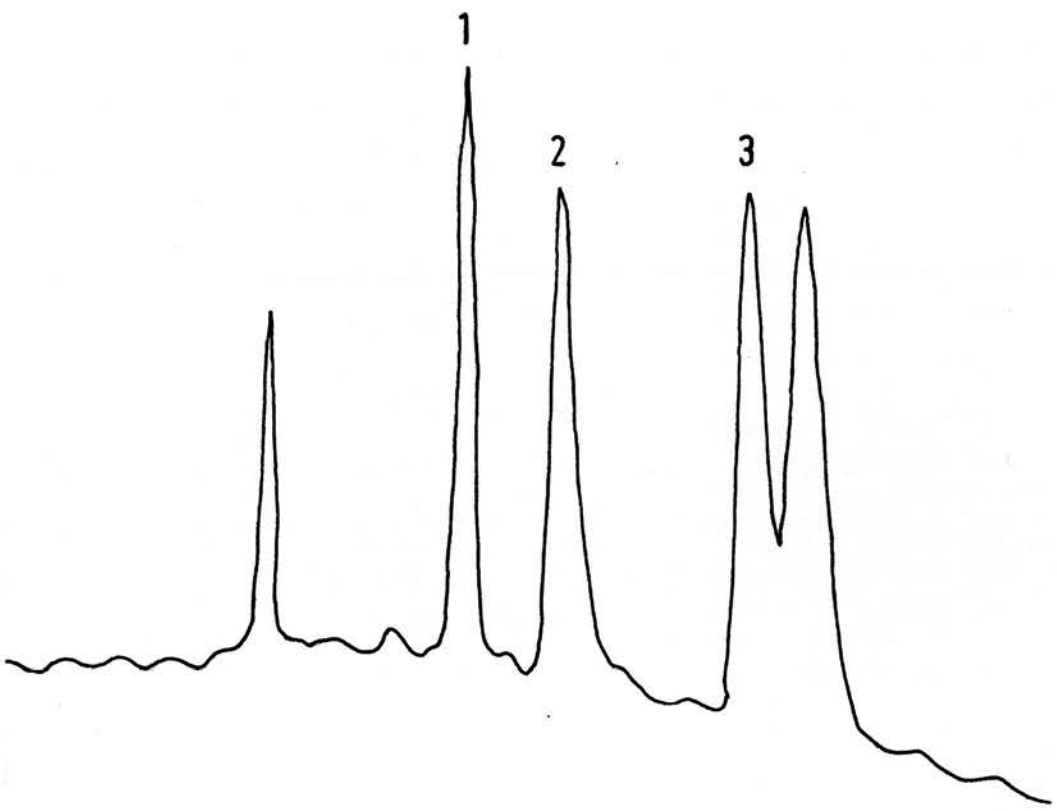


Figure 3

HPLC trace for tamoxifen metabolites.



1 4-OHT peak

2 DMT peak

3 TAM peak

Calculation

1. A figure is given for the 'area under the curve' on the plot produced from the integrator from which the actual level of tamoxifen or metabolite is calculated.

2. Because extraction efficiencies for each metabolite were not 100% an efficiency factor was required (to obtain 100% efficiency) and metabolite concentration was then calculated. This was calculated from accuracy and precision data from the standard curve. Accuracy was how near expected values were to observed values and precision was day-to-day variance in observed values. These data give values for the efficiency factors which were used in the final calculation along with the equations for line of best fit.

   The efficiency factors used for each metabolite are as follows:-

4OHT	1.59
DMT	1.64
TAM	1.69

3. Concentration (y) = mean area under the curve (a) x efficiency factor (e).

4. Calculate (x) which is the amount (concentration) of metabolite in 20µl of the original plasma sample using the standard curve equations and multiply (x) by 50 to obtain a value in ng/ml of plasma. A worked example is shown below.

   Standard Curve Equations

4-OHT	$\log x =$	$\frac{\log y - 4.3632}{1.114}$
DMT	$\log x =$	$\frac{\log y - 4.644}{0.96096}$
TAM	$\log x =$	$\frac{\log y - 4.6493}{1.0312}$

Worked Example

Result from patient MH

Results	4OH	DMT	TAM	CIS
Standards (mixed)	120000	220000	220000	300000
Standard (CIS)	-	-	-	300000
Effic factors (e)	1.59	1.64	1.69	1.56
Plasma	255390	246680	112320	-
Plasma + CIS	332550	324150	136840	190490*
Mean (a)	293970	285415	124580	

* this value was used to check the efficiency of each run (190490/300000 x 100 = 62.8%. If <50% then the sample result was discarded)

Measuring 4OH for patient MH

$y = a \times e$

$y = 293970 \times 1.59$

$y = 467412$

Place this into the standard equation

$\log x = \log y - 4.3632 / 1.114$

$\log x = \log (467412) - 4.3632/1.114$

$x = 2.97$ (ng in 20µl of sample)

$50x = 148.9$ng/ml

Results

Plasma Levels

Blood samples were taken from all 357 study patients, spun down and stored for analysis as previously described. Extraction was carried out on 333 samples due to time constraints and only results with recovery rates of over 50% were included for analysis. 160 results were available for plasma tamoxifen and desmethyltamoxifen and 152 for 4-hydroxytamoxifen. Total tamoxifen was therefore calculated from the 152 patients where results were available for all 3 metabolites.

The results for plasma levels measured are shown below:

	Tamoxifen (ng/ml)	**4-Hydroxytamoxifen (ng/ml)**	**Desmethyltamoxifen (ng/ml)**	**Total (ng/ml)**
Mean	66.5	47.4	115.2	235.0
SD	44.5	32.5	88.0	155.9
Median	52.6	38.1	85.0	187.8
Range	12.2-277.5	4.1-160.8	10.4-492.1	34.7-892.2

Endometrial Thickness

Plasma levels of tamoxifen (TAM), 4-hydroxytamoxifen (4OHT), desmethyltamoxifen (DMT) and total metabolites (the sum of all three) were compared with endometrial thickness as assessed by transvaginal ultrasound scan and the results for patients where extraction rates were >50% are as follows:

Metabolite	Endometrial Thickness
TAM	p=0.077
4-OHT	p=0.114
DMT	p=0.058
Total	p=0.114

Figures 4, 5, 6 and 7 demonstrate this graphically.

From the graphs it can be seen that there was a positive correlation between all the metabolites and endometrial thickness although none reached statistical significance. The relationship between endometrial thickness and both plasma tamoxifen and desmethyltamoxifen was of borderline significance.

Despite the positive correlation there was a wide scatter of points around the line making it difficult to extrapolate clinical management decisions from these results.

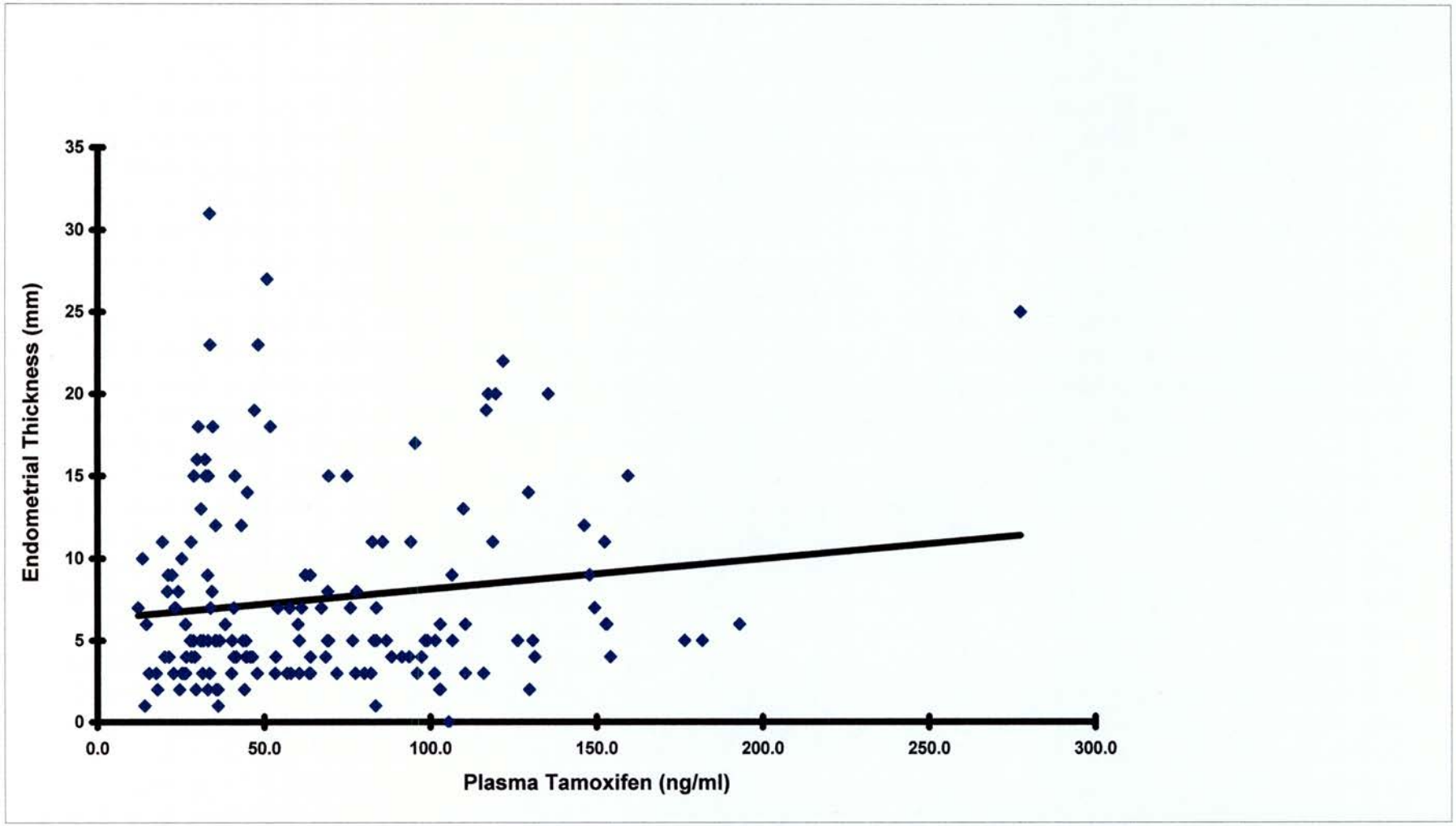


Figure 4
Relationship between plasma tamoxifen levels and endometrial thickness.

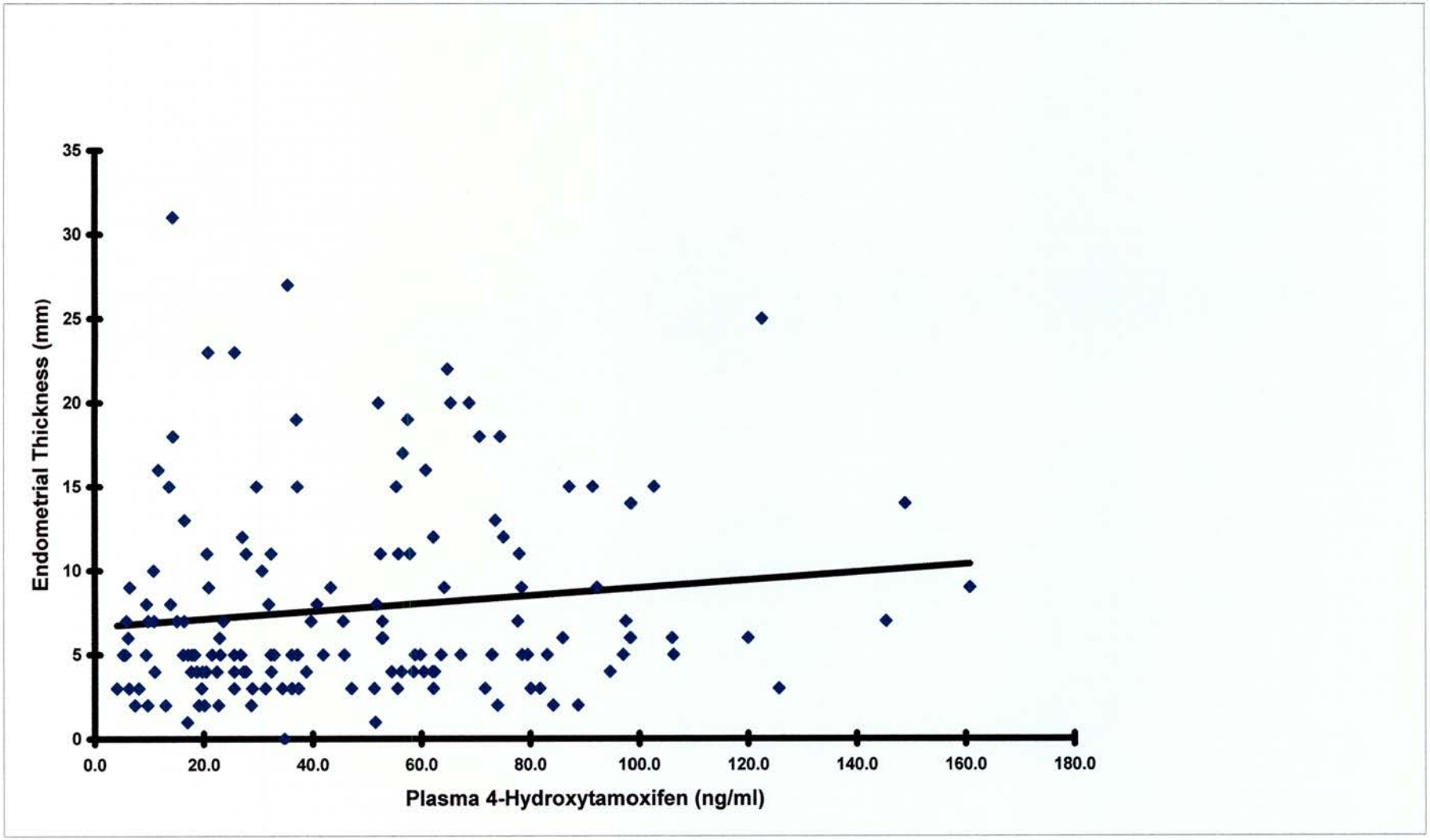


Figure 5
Relationship between plasma 4-hydroxytamoxifen levels and endometrial thickness.

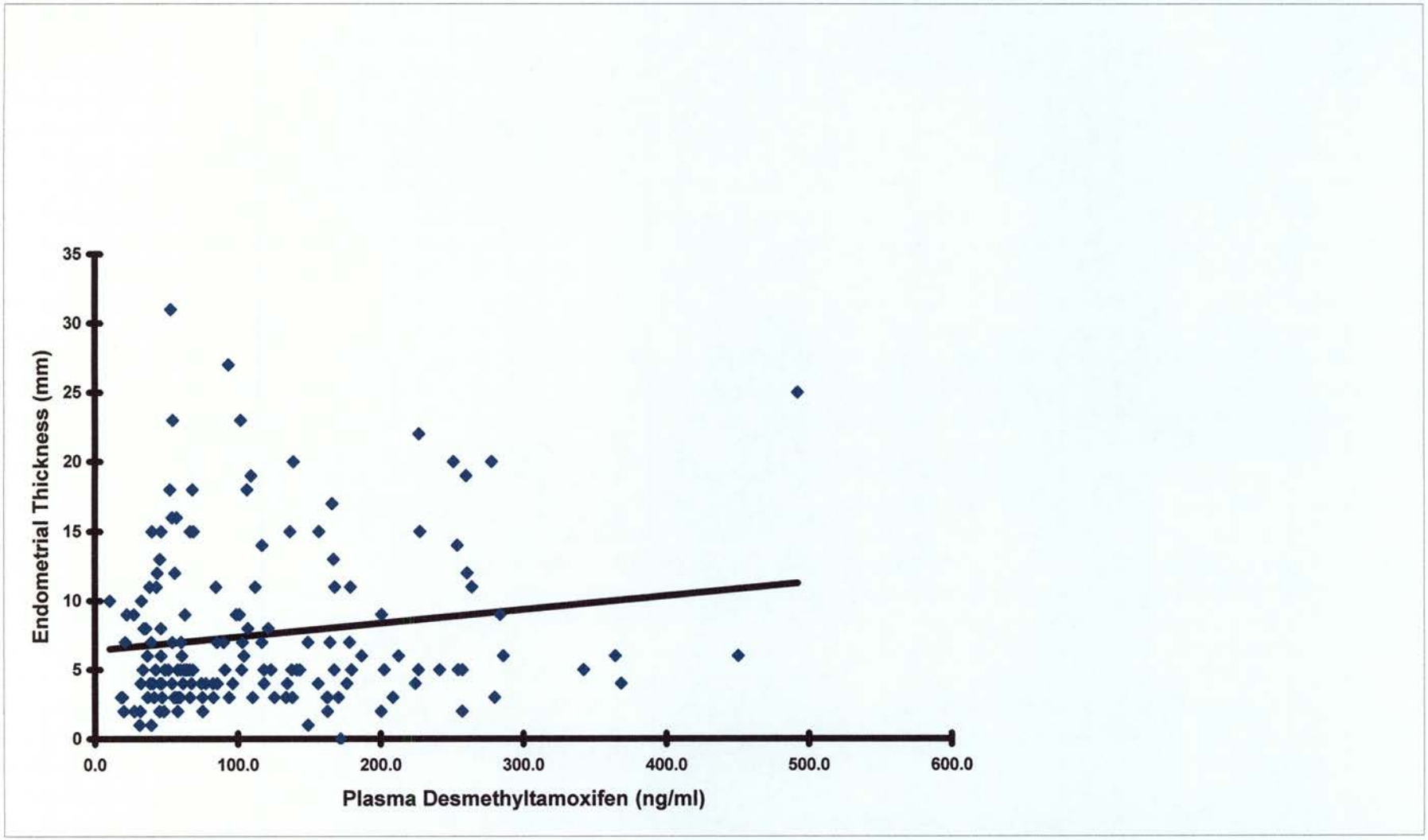


Figure 6
Relationship between plasma desmethyltamoxifen and endometrial thickness.

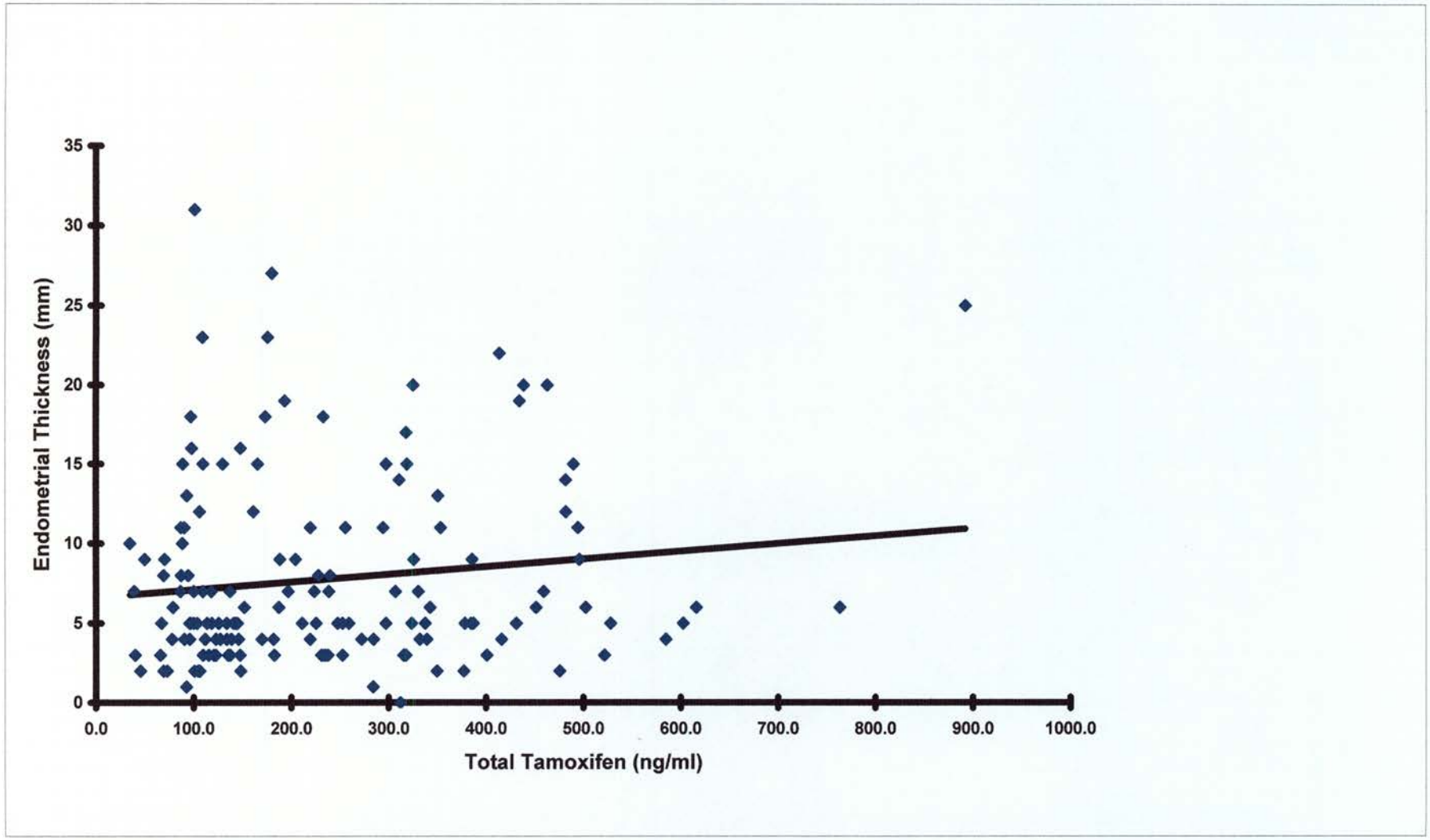


Figure 7
Relationship between total tamoxifen levels and endometrial thickness.

Body Mass Index

Each patient had height and weight recorded at the time of their first visit and body mass index (BMI) recorded using the following standard calculation.

$$BMI = \frac{\text{weight (kg)}}{\text{height (m}^2\text{)}}$$

A BMI of <25 is normal whereas >30 indicates obesity.

Thereafter the relatonship between BMI and plasma tamoxifen and its metabolites was investigated. The results are summarised below:

	Body Mass Index (SD)			
Metabolite (ng/ml)	<25	25-30	>30	
TAM	66.2(41.3)	70.0(49.9)	58.9(39.3)	p=0.522
4-OHT	50.3(32.4)	46.6(32.1)	38.9(25.9)	p=0.045
DMT	117.9(89.8)	120.6(95.0)	97.2(65.6)	p=0.349
Total	245.7(156.1)	241.1(168.9)	194.9(121.7)	p=0.177

These results are demonstrated graphically in Figures 8, 9, 10 and 11.

There was a significant negative correlation between body mass index and plasma 4-hydroxytamoxifen. Therefore, as BMI increases, levels of 4-hydroxytamoxifen appear to fall. The correlations between body mass index and tamoxifen, desmethyltamoxifen and total tamoxifen failed to reach statistical significance.

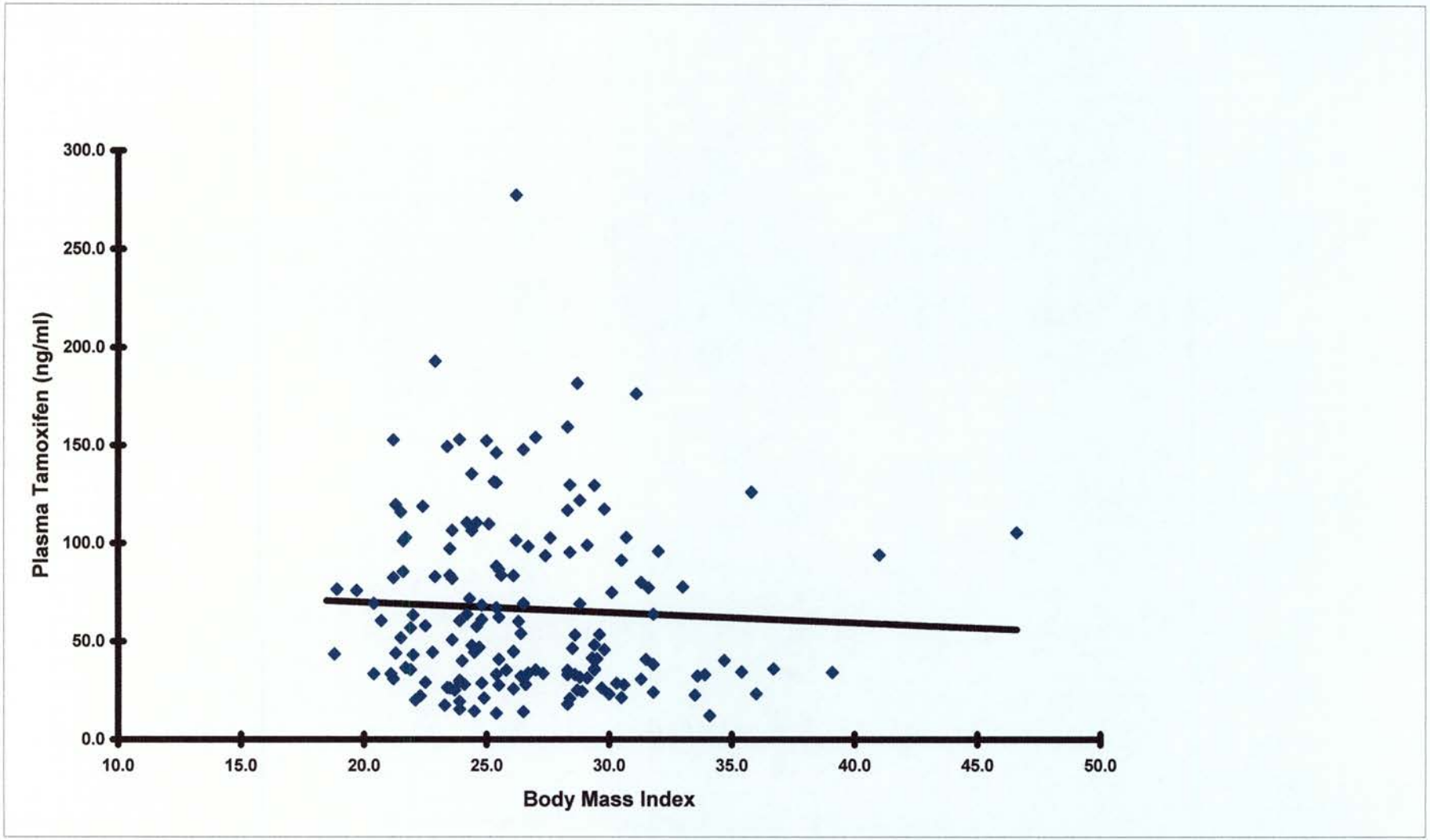


Figure 8
Relationship between BMI and plasma tamoxifen levels.

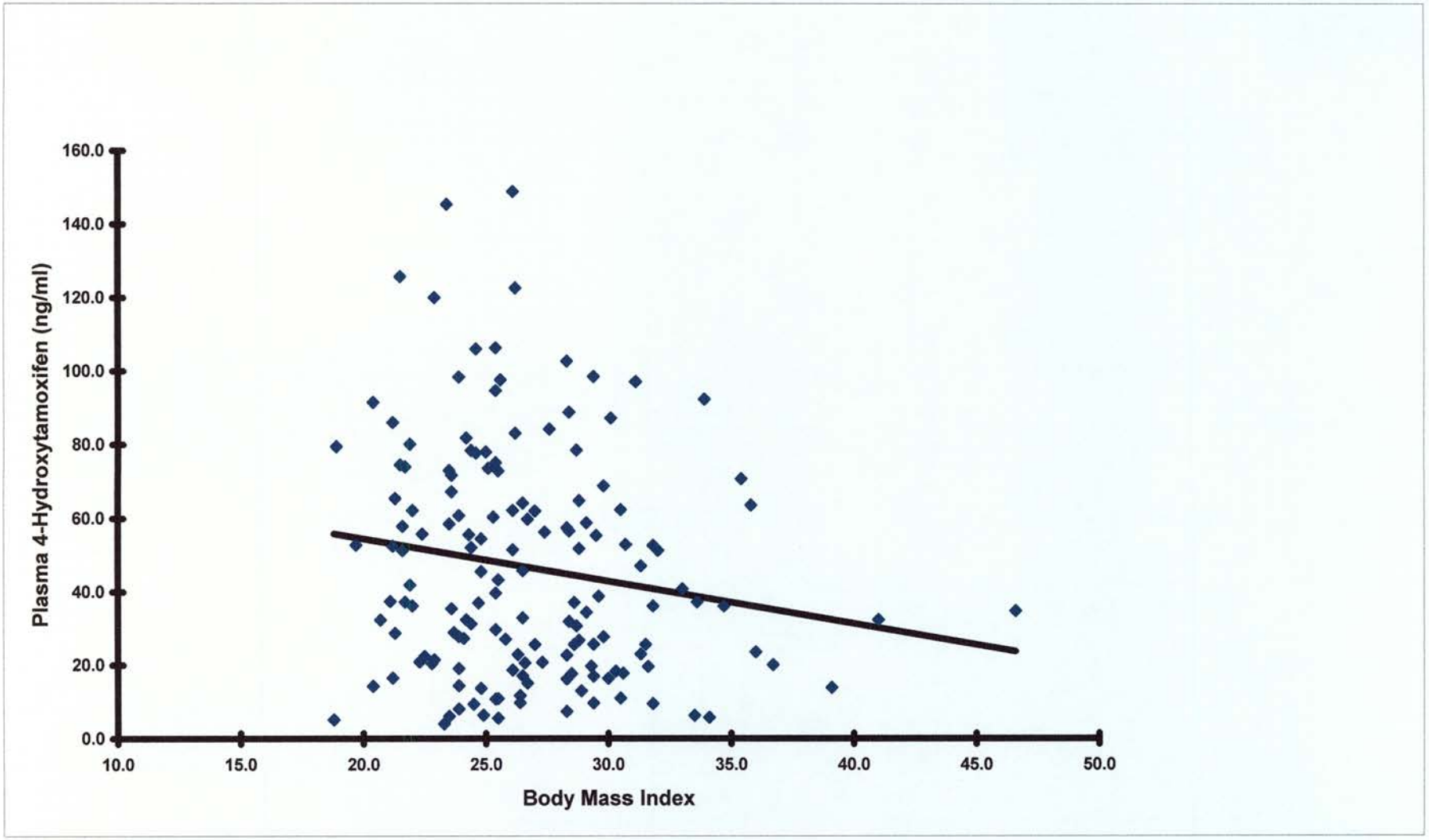


Figure 9
Relationship between BMI and plasma 4-hydroxytamoxifen.

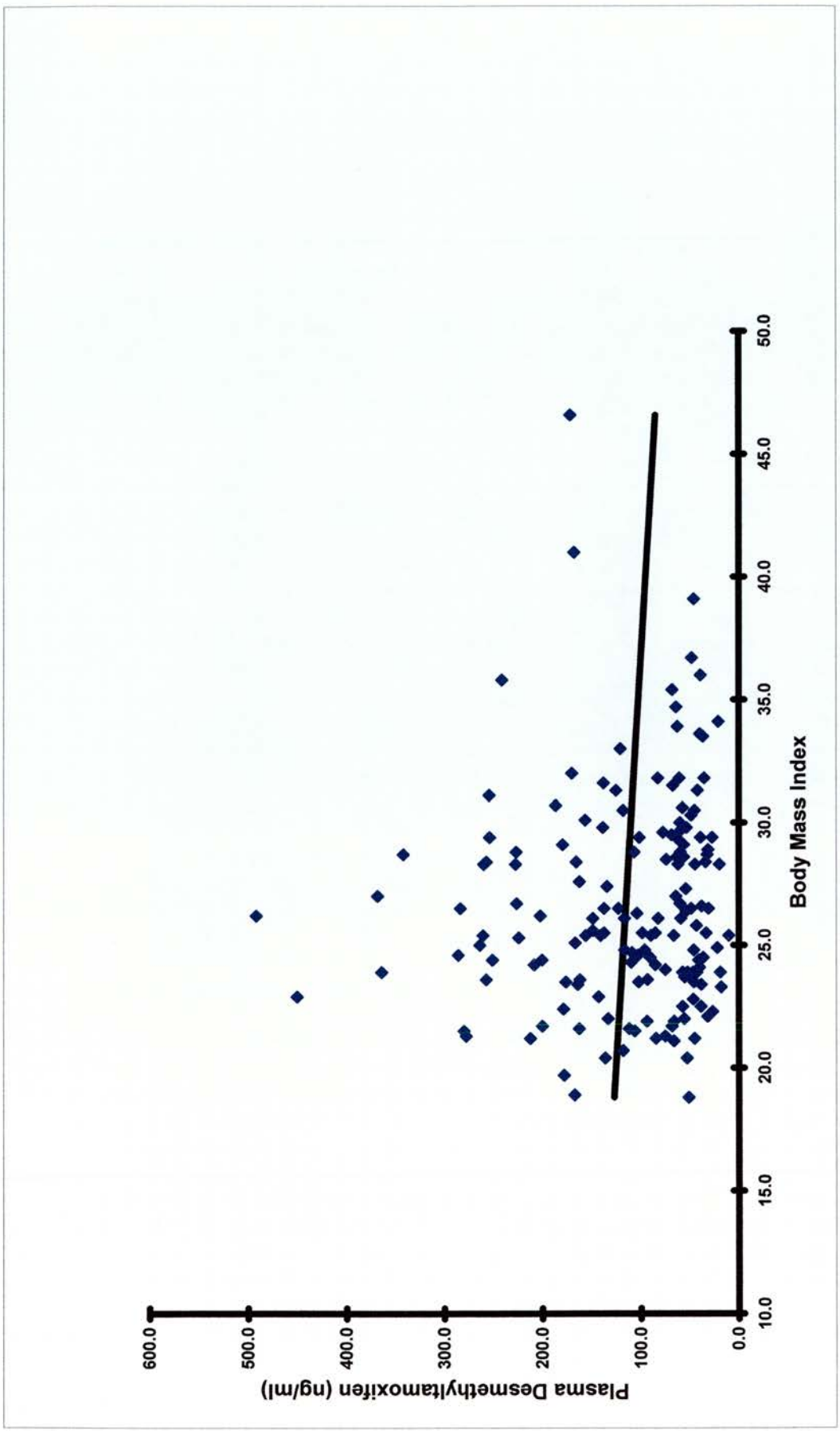


Figure 10
Relationship between BMI and plasma desmethyltamoxifen levels.

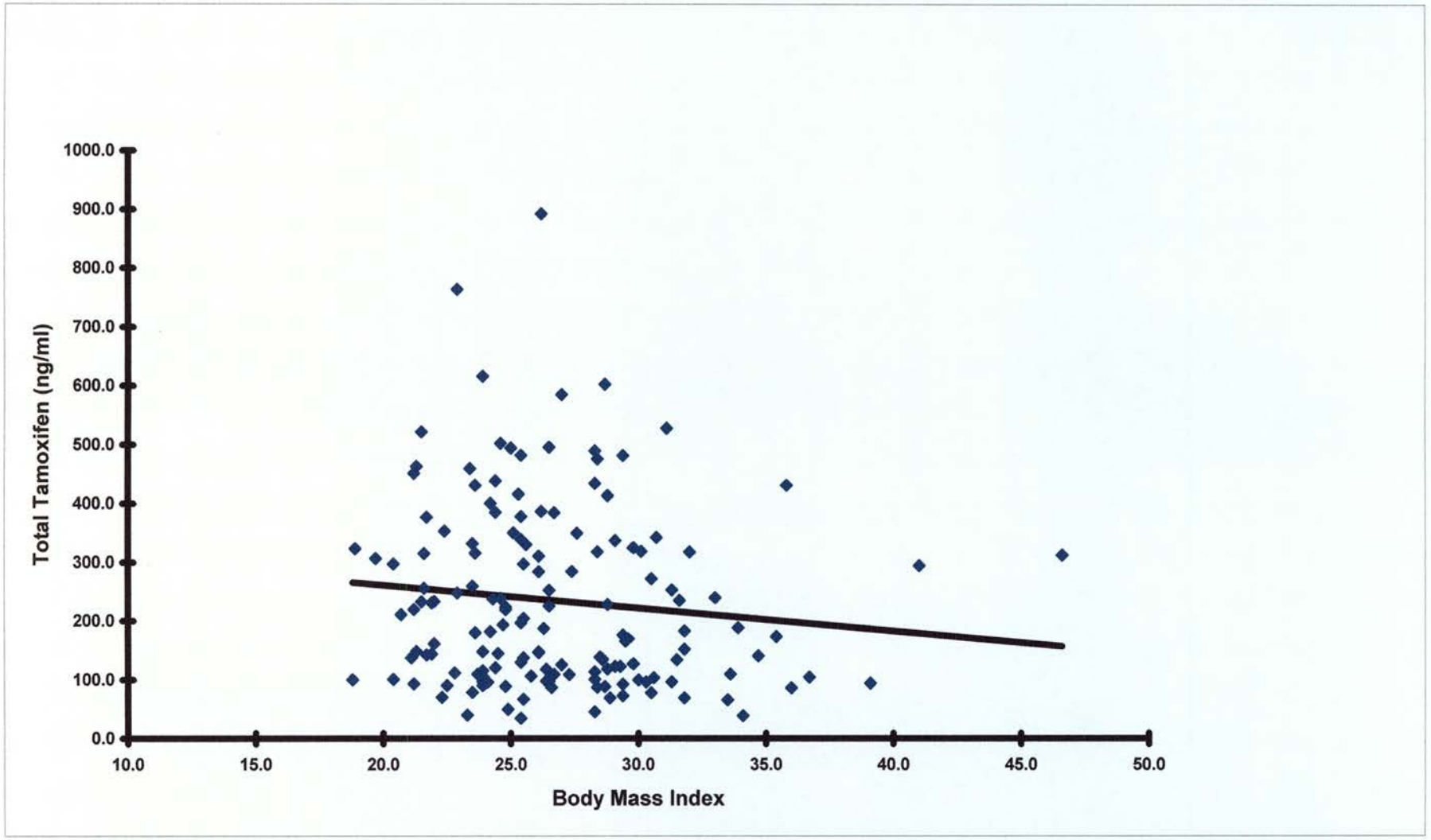


Figure 11
Relationship between BMI and total plasma tamoxifen levels.

Discussion

The wide variation in plasma levels of tamoxifen and its metabolites are well recognised. Lien et al reported mean plasma tamoxifen levels of 163ng/ml (range 79-299), desmethyltamoxifen levels of 199ng/ml (range 76-410) and 4-hydroxytamoxifen levels of 4.2ng/ml (range 0.7-7.1) in patients receiving 30mg/day tamoxifen. Although this group received a greater dose of tamoxifen than this study population, studies in the literature agree that the daily dose of tamoxifen is largely irrelevant with regard to plasma levels and does not play a role in the wide patient variation observed [Robinson 1994, Osborne 1998, Ross 1995]. Robinson [1994], in a study of 20mg/day reported mean plasma levels of 108ng/ml for tamoxifen, 238ng/ml for desmethyltamoxifen and 2.8ng/ml for 4-hydroxytamoxifen and MacCallum [1997] of 30ng/ml for tamoxifen, 190ng/ml DMT and 54ng/ml for 4-OHT. Concentrations of desmethyltamoxifen are generally higher and 4-hydroxytamoxifen very low. The present study population also exhibited a wide range of plasma levels in keeping with the literature although mean plasma 4-OHT levels of 47.4ng/ml (range 4.1-160.8) were higher than most other reports apart from MacCallum et al which report similarly high levels and may have occurred because the same method was used. This wide plasma variation did not appear to follow a normal distribution, however, as one might have expected.

There is no satisfactory explanation for the wide variation in plasma levels seen. Tamoxifen has a long half-life (7 days) and desmethyltamoxifen even longer (14 days). Steady state is achieved after 30 days and serum levels have been shown to be stable long term - for at least 10 years of therapy. There are no significant peak and trough differences in plasma levels and variations observed between individuals are not usually seen in serial samples from the same patients. Most studies report no metabolic tolerance developing [Langan-Fahey 1990, Fabien 1980, Jordan 1990, Lerner 1990, MacCallum 1996, Osborne 1998]. Fabien suggested that large variations in individual patient sampling may be a result of variation in drug absorption and metabolism as influenced by other medications and hepatic and renal function but this has yet to be confirmed.

Langan-Fahey's [1990] study is the only one to assess whether the high individual variation in plasma levels is related to obesity. Levels of tamoxifen and desmethyltamoxifen were

found not to correlate with indices of obesity. The present study confirmed these findings but did find a significant negative correlation between plasma 4-hydroxytamoxifen and body mass index. So as BMI rises, levels of 4-OHT fall. In isolation, the clinical significance of this finding is uncertain.

Significant differences in tamoxifen and metabolite levels have also been reported in tissue, both breast tumour and other and do not correspond with responding and resistant tumours either but may perhaps reflect tumour heterogeneity [Lerner 1990, MacCallum 1997]. Studies have been performed investigating tamoxifen and metabolite levels in the rat and mouse uterus. Robinson [1991] found a greater accumulation of tamoxifen, desmethyltamoxifen and 4-hydroxytamoxifen in liver and uterus following very large oral doses and there was a higher concentration of both tamoxifen and DMT compared with blood in the rat uterus, more than 100x greater. One suggested explanation for this observation was the presence of high affinity anti-oestrogen binding sites within the liver and uterus but more recent literature would not support this theory [Robinson 1994]. Studies have yet to be reproduced investigating tamoxifen and metabolite levels in human endometrium and whether these correlate with endometrial pathology. The present study aimed to correlate plasma levels with endometrial abnormalities but when limited pathology was found, the aim was altered to correlate plasma levels with endometrial thickness on ultrasound scan. There was no significant correlation between plasma levels and endometrial thickness although a correlation with plasma tamoxifen and desmethyltamoxifen almost reached statistical significance. This information is of limited use as endometrial thickness on ultrasound scan has been shown not to correlate well with pathology (46% false positive scan rate in women on tamoxifen). In addition, there was no relationship between plasma 4-hydroxytamoxifen (the most oestrogenic of the metabolites measured) and endometrial thickness which one may have anticipated in view of tamoxifen's oestrogenic action on human endometrium.

AROMATASE INHIBITOR - LETROZOLE

Introduction

Letrozole or CGS 20 267, 4,41-(1H-1,2,4-Triazol-l-ylmethylene)bis-bezonitrite is one of the third generation oral aromatase inhibitors. It is a potent and highly specific non-steroidal inhibitor in vivo and has been found to be 200 times more potent than aminoglutethamide in-vitro and more selective [CGS 20 267 1988]. Because of the concerns regarding the potentially serious side effects of tamoxifen newer, more specific, endocrine agents including letrozole have been under investigation as alternative therapies. In Phase 1 trials letrozole was found to significantly suppress oestrone and oestradiol levels peripherally and showed antitumour activity in some patients resulting in an overall clinical response rate of 33% [Iveson 1993].

Treatment with aromatase inhibitors is aimed at achieving maximal oestrogen suppression with minimum toxicity. In Phase 1 trials, doses of 0.1mg, 0.5mg and 2.5mg letrozole all produced significant oestrogen suppression (95%) but there was a tendency for increasing doses to produce increasing suppression (although this was not statistically significant). In addition, over a wide dose range, letrozole does not significantly change levels of gonadotrophins, ACTH, cortisol, aldosterone or TSH supporting its selectivity for the inhibition of aromatase alone.

Studies of postmenopausal women with metastatic breast cancer who progress on tamoxifen have shown an overall survival advantage for patients receiving letrozole over megestrol acetate or aminoglutethamide [Santen 1999] and aromatase inhibitors are now being used in adjuvant trials to determine the efficacy of aromatase inhibitors versus tamoxifen versus combination of antioestrogen and aromatase inhibitor.

This study aimed to investigate the effectiveness of 2 doses of letrozole (2.5mg and 10mg) as primary medical therapy (neo adjuvant) for patients with locally advanced and large operable breast cancer. Neoadjuvant therapy has been used to treat large operable and locally advanced breast cancers but studies to date have concentrated mainly on the use of chemotherapy. A few centres have assessed endocrine agents in this setting, of which the

most commonly used is tamoxifen, but this is the first study to assess the third generation aromatase inhibitor, letrozole, as neoadjuvant treatment.

Patients and Methods

24 postmenopausal patients (age range 53-87 years) were treated in sequence, the first 12 received 2.5mg of letrozole, the second 12 were treated with 10mg of letrozole. All patients had oestrogen receptors assayed on tumour biopsy specimens using either an enzyme immunoassay (ER-EIA) using the Abbott kit, according to the manufacturer's instruction or an enzyme immunochemical assay on immunohistochemistry. Patients were classed as oestrogen receptor positive if they had greater than 20fmol receptor/mg/cytosol protein or a histoscore of >80 (histoscore calculated by multiplying the percentage of cells staining by the intensity of the staining graded from 0-3) [McCarty 1985].

Breast cancers are staged using the TNM classification (Tumour size - Node - Metastasis) as follows:

Tis	in situ
T1	<2cm
T2	>2-5cm
T3	>5cm
T4a	Involvement of chest wall
b	Involvement of skin
c	a and b together
d	Inflammatory cancer
N 0	No regional node metastasis
N 1	Mobile ipsilateral nodes
N 2	Fixed ipsilateral nodes
N3	Internal mammary node involvement
M 0	No evidence of metastasis
M 1	Distant metastasis

Only patients with large operable or locally advanced breast cancers without evidence of metastases were included in this study, T2>3cm, T3 T4b, N0-1, M0. Patients were M0 on the basis of normal biochemistry and no metastases on a chest x-ray or bone scan. All 24 patients gave written informed consent.

At the outset of the study all tumours were measured clinically using engineers' calipers in four different directions 45° apart and the tumour volume calculated using the formula:

$$V = \frac{D^3 \times \pi}{6}$$

(V: volume, D: mean diameter)

They also had mammograms and using the measurements on the oblique and craniocaudal views the largest tumour diameter and the diameter at 90° to the axis was measured. The mean mammographic diameter was calculated and tumour volume calculated using the formula:

$$V = \frac{D^3 \times \pi}{6}$$

(V: volume, D: mean diameter)

Patients also had a breast ultrasound and four scans performed at 45° apart using the machine's electronic callipers and tumour volume was estimated according to the following formula:

$$\underline{V = D^2 \times d \times \pi}$$
$$6$$

(V: volume, D: mean diameter, d: mean thickness)

All methods of measurement have been described by Forouhi et al [1994].

Patients receiving 10mg of letrozole also had a short synacthen test performed prior to starting treatment and after completion of three months' treatment with this dose of letrozole. This was to confirm that higher doses of letrozole had no effect on steroid levels.

Patients were treated with letrozole for three months. During this time they were monitored at monthly intervals and had clinical and ultrasound tumour volumes calculated at each visit.

A second mammogram was performed at the end of the three months and mammographic volumes calculated. Percentage change in tumour volume was used to assess tumour response. Modified WHO criteria were used, a partial response (PR) being defined as a 50% or greater reduction in tumour volume and a complete response (CR) being no measurable tumour. Progression of disease (PD) was defined as greater than 25% increase in tumour volume with no change (NC) being defined as a volume decrease of less than 50% or a volume increase of no greater than 25%.

Results

All 24 patients completed the three month course of neo-adjuvant therapy. The only reported side effect was in one patient who was taking 2.5mg of letrozole and reported transient pale stools. A second patient taking 10mg of letrozole had low platelet levels at the end of treatment. Subsequent investigation demonstrated that this patient had bone marrow involvement which had not been detected by any pre-operative investigations.

There were no abnormalities in any of the synacthen tests indicating that even at the 10mg dose there was no effect on adrenal corticosteroid production. All bone scans performed prior to and following treatment were normal.

Letrozole 2.5mg

Table 1 shows the clinical tumour volumes at 0 and 3 months and the % reduction in tumour volumes and Table 2 the imaging response (% reduction in volume on ultrasound and mammography).

Figure 1 shows the changes in tumour volumes at 3 months as assessed by clinical examination, mammography and ultrasound.

On the basis of the modified WHO classification, of the 12 patients treated by 2.5mg of letrozole there were five complete clinical responses and seven partial responses. Results based on the best imaging response were 1 complete response, 10 partial responses and 1 no change.

Letrozole 10mg

Table 3 shows the clinical tumour volumes at 0 and 3 months and the % reduction in tumour volumes and Table 4 the imaging response. Figure 2 shows the same data as above for the 12 patients who took 10mg letrozole.

There were 2 complete clinical responses, 8 partial responses with 2 patients being classified as having stable disease. Best imaging responses were 11 partial responders and 1 no change.

When comparing the results of 2.5mg and 10mg letrozole, there were no apparent differences between responses for these two doses. All but two patients had a greater than 50% reduction in clinical tumour volume.

Nine patients were suitable for breast conservation at the outset of treatment but by the end of the three month course of letrozole all patients were eligible for treatment by breast conservation. Histology demonstrated that all cancers were completely excised. There was one complete pathological response in a patient treated by 2.5mg letrozole and three patients had microscopic disease only, two in the 2.5mg and one in the 10mg group.

Figure 3 shows the mammographic change from 0 to 3 months in the patient who had the complete pathological response.

Patient	Age	Maximum tumour diameter (cm)	Clinical tumour volume time 0	Clinical tumour volume 3 mth	% Reduction in clinical tumour volume	Clinical response	Tumour dimensions (cm)	Pathological volume at surgery
1	86	5.0	40.2	0	100	CR	1.2 x 1.3 x 1.1	0.90
2	75	3.5	17.9	0	100	CR	2.5 x 1.0 x 0.7	0.92
3	78	4.2	18.8	2.5	86.7	PR	1.0 x 1.0 x 1.3	0.68
4	87	5.0	13.4	0	100	CR	no residual tumour	0.08
5	84	4.6	50.9	10.6	79.2	PR	2.2 x 2.4 x 3.0	8.29
6	81	3.0	9.2	0.6	93.5	PR	1.1 x 0.9 x 1.0	0.52
7	80	5.0	34.1	10.3	69.8	PR	3.5 x 2.4 x 0.5	2.20
8	68	3.8	22.4	6.6	70.5	PR	2.0 x 0.7 x 1.4	1.03
9	61	4.5	36.1	5.7	84.2	PR	3.0 x 2.2 x 2.1	7.25
10	85	4.3	23.9	0	100	CR	1.5 x 1.0 x 1.0	0.86
11	62	5.1	59.7	16.7	72	PR	2.4 x 2.0 x 2.0	5.02
12	75	5.8	37.4	0	100	CR	0.7 x 0.7 x 0.7	0.18

Table 1

Patients treated with 2.5mg of letrozole.

One patient did not have an initial mammogram because her tumour was locally advanced and it was not possible to obtain an adequate mammogram.

No	Mammo tumour volume time 0	Mammo tumour volume time 3 mth	% Reduction in mammo volume	US volume time 0	US volume 3 mth	% Reduction in US volume	Best imaging response
1	14.1	5.2	63.1	6.6	3.5	47	PR
2	4.8	0.3	93.7	1.4	1.4	0	PR
3	20.6	5.9	71.4	4.4	1.9	56.8	PR
4	41.6	0.1	99.8	7.1	0.3	95.8	PR
5	-	5.9	-	4.8	3.2	33.3	NC
6	6.4	1.1	82.8	3.1	0.6	80.6	PR
7	43.1	17.9	58.5	12.8	2.4	81.3	PR
8	12.1	2.8	76.9	8.9	1.5	83.1	PR
9	27.6	2.6	90.6	8.4	2.7	67.9	PR
10	37.4	3.6	90.4	7.1	1.2	83.1	PR
11	37.4	17.9	48.4	14	6.7	52.1	PR
12	7.7	0	100	6.4	0.3	95.3	CR

Table 2

Patients treated with 2.5mg of letrozole.

No	Age	Maximum tumour diameter (cm)	Clinical tumour volume time 0	Clinical tumour volume 3 mth	% Reduction in clinical tumour volume	Clinical response	Tumour dimensions (cm)	Pathological volume at surgery
1	80	4.0	17.8	3.7	79.1	PR	1.0 x 1.0 x 0.8	0.42
2	53	4.0	25.5	7.9	59.0	PR	1.2 x 1.7 x 1.8	1.92
3	78	3.7	22.9	6.2	68.7	PR	2.2 x 1.5 x 0.2	0.35
4	65	3.7	18.8	0	100	CR	Nil	0.0
5	71	3.8	24.9	2.9	88.4	PR	1.2 x 0.9 x 0.6	0.34
6	74	5.3	53.5	8.2	84.7	PR	1.5 x 1.2 x 1.0	0.94
7	82	5.5	71.5	16.4	77.1	PR	1.8 x 1.5 x 1.3	1.84
8	61	4.3	28.7	33.5	-16.7	SD	2.0 x 3.0 x 1.0	3.14
9	68	4.8	36.7	18.3	50.1	PR	2.0 x 2.0 x 2.0	4.19
10	76	5.0	48.5	0	100	CR	1.3 x 1.2 x 1.0	0.82
11	69	4.1	31.0	18.0	41.9	SD	1.2 x 0.8 x 0.8	0.40
12	74	6.0	59.7	18.4	69.2	PR	1.5 x 1.7 x 2.0	2.67

Table 3

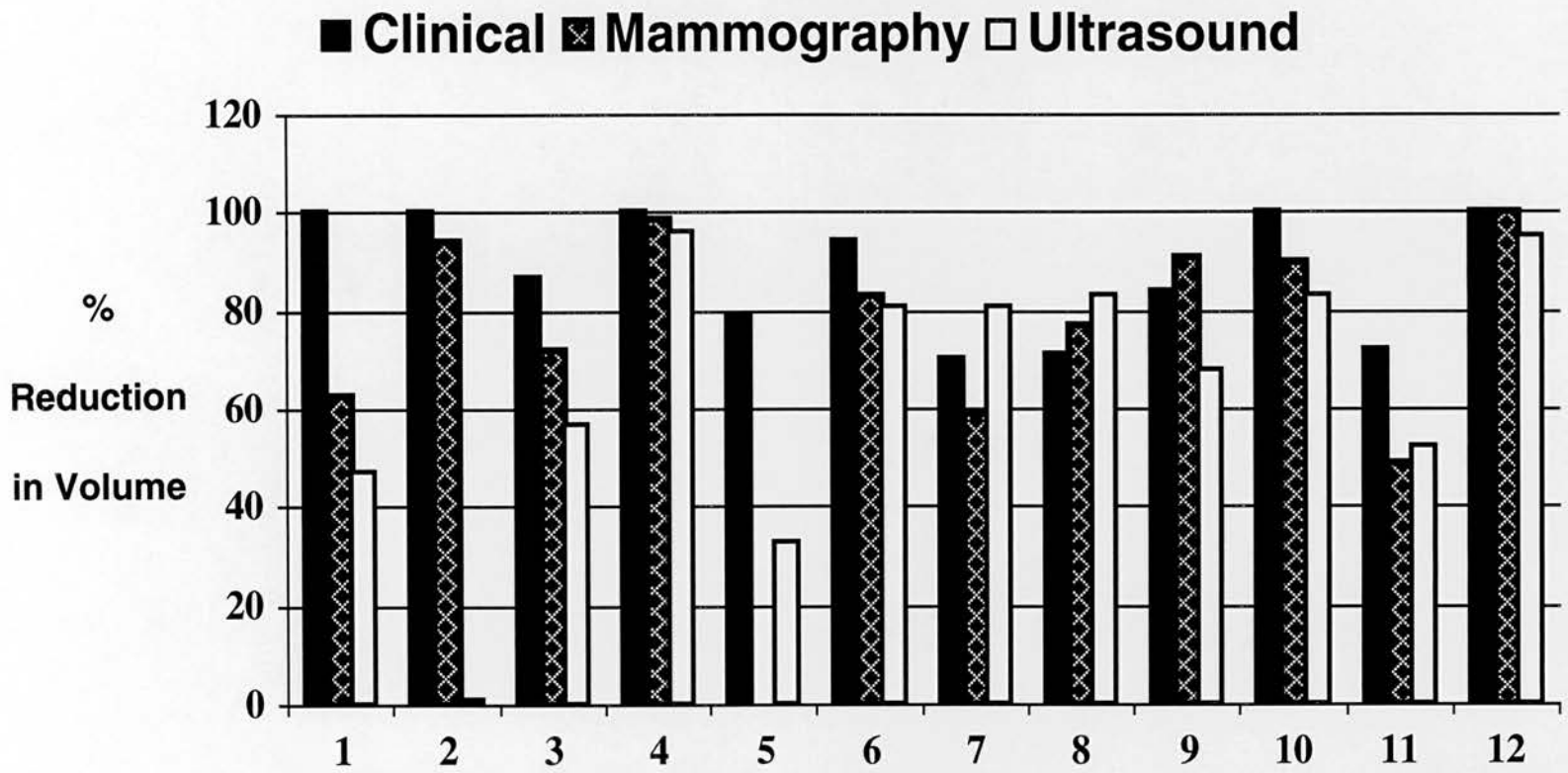
Patients treated with 10mg of letrozole.

No	Mammo tumour volume time 0	Mammo tumour volume time 3 mth	% Reduction in mammo volume	US volume time 0	US volume 3 mth	% Reduction in US volume	Best imaging response
1	17.9	2.8	84.4	2.3	1.11	52.4	PR
2	10.9	1.8	83.5	3.1	0.94	69.5	PR
3	12.8	8.68	32.2	7.5	3.02	59.8	PR
4	20.6	2.8	86.4	11.4	0.18	98.4	PR
5	8.2	1.8	78.6	3.9	2.39	39	PR
6	14.1	5.2	63.1	12.9	2.39	81.5	PR
7	27.6	2.8	89.9	8.2	1.52	81.5	PR
8	10.9	8.2	24.8	3.2	6.09	89.1↑	NC
9	31.0	7.2	76.8	13.6	2.27	81.9	PR
10	14.1	4.8	66	3.7	1.29	65.4	PR
11	3.31	1.1	66.8	3.9	0.6	84.06	PR
12	13.44	4.8	64.3	8.5	3.2	62.3	PR

Table 4

Patients treated with 10mg of letrozole.

Figure 1
Clinical, mammographic and ultrasound changes in tumour volume at 3 months in 12 patients treated with Letrozole 2.5mg



One patient, patient number 5, did not have a mammogram at the outset because the tumour was locally advanced and was unsuitable for initial mammography

Figure 2
Clinical, mammographic and ultrasound changes in tumour volume at 3 months in 12 patients treated with Letrozole 10mg

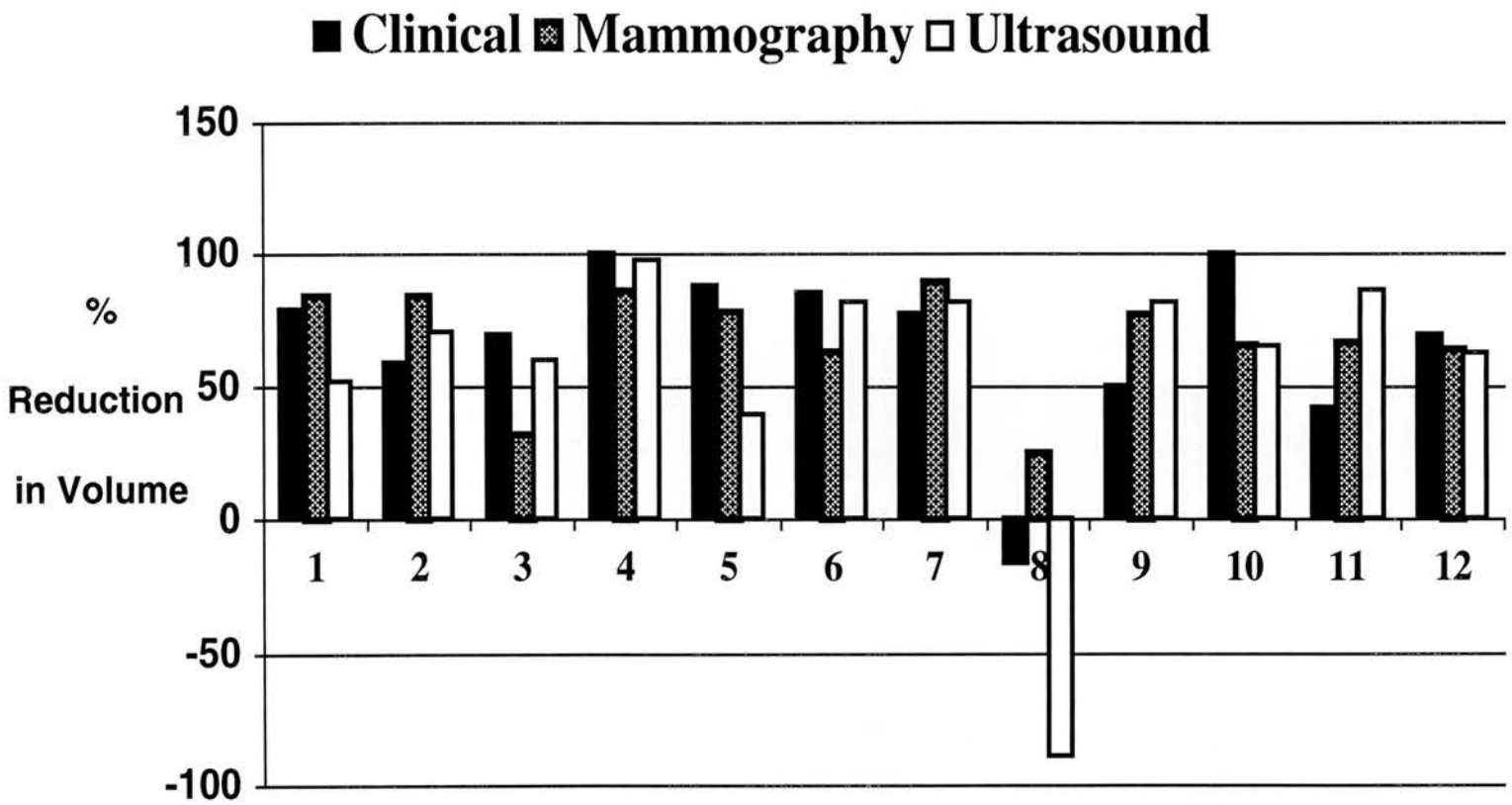
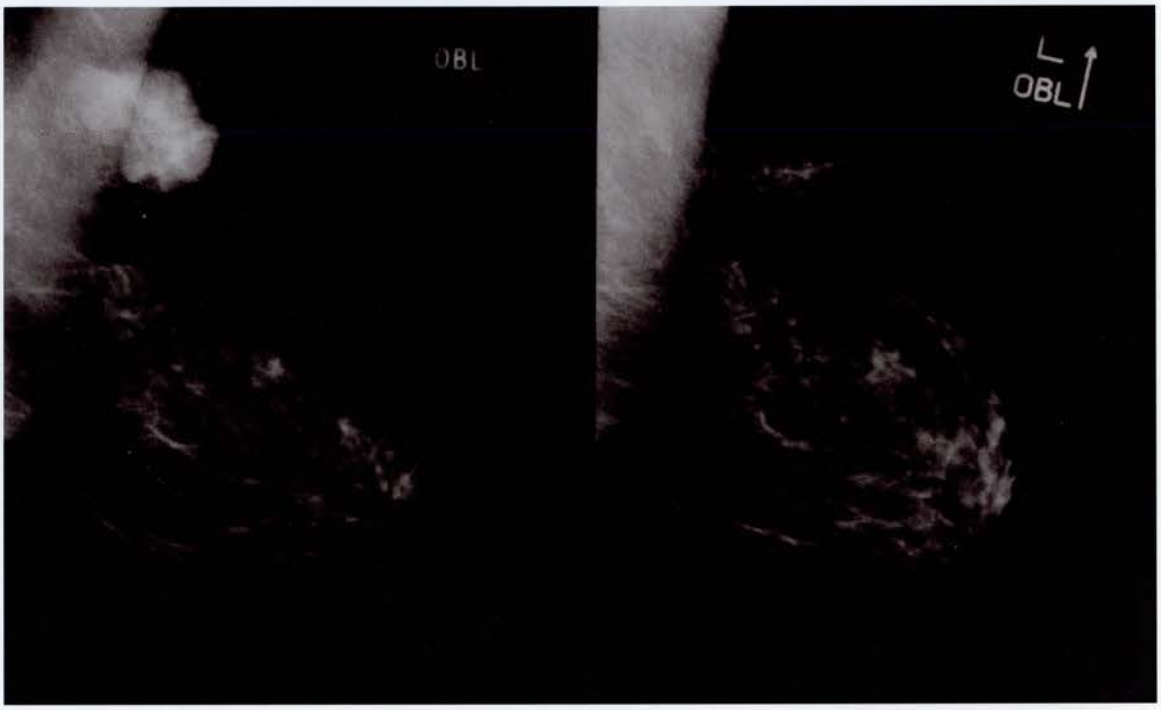


Figure 3

Mammograms of breast malignancy pre and post letrozole treatment.



PRE LETROZOLE **POST LETROZOLE**

Discussion

Neo-adjuvant therapy has to date consisted of primary chemotherapy or primary endocrine therapy in the form of tamoxifen. Indirect comparisons in the World Overview indicate that survival using adjuvant tamoxifen as primary endocrine therapy appears equivalent to chemotherapy in young women and superior to chemotherapy in older women with oestrogen receptor positive tumours. Since the benefits of chemotherapy are unknown in women over 70 years, endocrine therapy is particularly important in this group which represent about a third of all breast cancers.

There have been several small trials investigating neo-adjuvant treatment with tamoxifen and Keen et al [1997] showed significant reductions in tumour volume over a 3 month period with tamoxifen (75% response in a group of postmenopausal women with high levels of oestrogen receptor). Response rates in this series with letrozole appear to be at least as good and possibly better than with tamoxifen, however, with 7/24 patients having a complete clinical response and 22/24 (92%) having a greater than 50% reduction in clinical tumour volume.

One of the aims of primary endocrine therapy is to reduce tumour volume in order to make breast conservation a feasible surgical option. Such have been the reductions in tumour volume in this study that all women were suitable for the less extensive surgery of wide excision following 3 months of letrozole therapy. From a cosmetic perspective this is advantageous and because many of these patients are elderly as, in this elderly population, mastectomy is associated with a 1% mortality [Hunt 1980]. Only 9/24 patients were suitable for breast conservation at the outset of treatment but by the end of the 3 month course of letrozole all women were eligible for breast conservation. Letrozole is at least as effective as tamoxifen in the neo-adjuvant setting and as it appears to be associated with less side effects than tamoxifen and no endometrial effects its role as neo-adjuvant treatment of elderly women with ER positive breast cancer needs further investigation.

Third generation aromatase inhibitors are being used in the management of tumours resistant to anti-oestrogens and should, in the long term, establish themselves as important drugs in the endocrine management of patients with breast cancer. Before tamoxifen can

be displaced as first line endocrine therapy, results from randomised clinical trials will need to show that these newer agents have comparable efficacy in this setting and have less toxicity. Even then it will be necessary to demonstrate that the benefits of using aromatase inhibitors are superior to those of conventional anti-oestrogens which have been used for over 25 years. Another option is to use aromatase inhibitors in combination with antioestrogens to produce complete oestrogen blockade but the disadvantage with this approach is that all methods of hormonal manipulation are used up front giving no further option if endocrine resistance develops. We know from clinical trials to date that third generation aromatase inhibitors block up to 90% of oestrogen production. Of concern, if these drugs are to be used in the adjuvant setting, is the effect of long term oestrogen suppression in a postmenopausal group with the potential morbidity from bone loss and cardiovascular complications. At present aromatase inhibitors have not been used in premenopausal women as there are very high levels of aromatase in the premenopausal ovary which are too high for blockade to be achieved by current clinical doses of the drug.

Also, aromatase inhibitors do not influence other areas of potential oestrogenic activity, for example $\Delta 5$ androgens, large quantities of which are produced in the adrenal cortex and which are capable of eliciting oestrogenic responses including stimulation of hormone dependent breast cancers. Nor will the aromatase inhibitors affect exogenously derived oestrogens (synthetic, industrial pollutents, phyto-oestrogens).

The role that aromatase inhibitors could play in the treatment of oestrogen dependent gynaecoloical disorders such as fibroids or endometriosis has not been fully investigated. The dose could be titrated against symptoms and complete oestrogen suppression which is produced using current treatments could be avoided. However, early data would suggest that aromatase inhibitors alone are not the answer as they appear to cause ovarian hyperstimulation in the premenopausal population.

There is no doubt that the third generation oral aromatase inhibitor, letrozole provides a promising alternative to conventional 'antioestrogens' such as tamoxifen used as first line treatment for breast cancer at least in the short term in terms of efficacy and lack of toxicity. Long term studies are required to show whether this effect is sustained and there are no problems with long term morbidity. Third generation aromatase inhibitors are being

investigated in the adjuvant setting and there are currently protocols in place to assess the gynaecological effects but results are several years away and perhaps a sound clinical model is required to assess its effects rather than waiting for results from ongoing adjuvant studies.

CONCLUSION

This study has focused on the endometrial changes secondary to tamoxifen use in breast cancer patients. Prior to commencement of the study the increased incidence of endometrial cancer and other endometrial pathology associated with tamoxifen use was regularly being reported and was of growing concern, but the question of whether screening women on tamoxifen was required had not been addressed. The main aim of this study was to answer this question and establish the best screening method if this was to be employed. It is one of the largest studies to prospectively assess both women on tamoxifen and controls and has shown conclusively that screening women on tamoxifen is not of benefit in terms of detecting abnormalities and therefore the cost to the patient and to the health service cannot be justified. Previous studies have reported high incidences of endometrial abnormalities in particular endometrial hyperplasia, which may be a precursor to endometrial cancer but this study which investigated asymptomatic women failed to confirm these findings and found no cases of hyperplasia or cancer in this population. Benign abnormalities found were in the form of submucous fibroids and endometrial polyps. All polyps removed were benign, but some have suggested that tamoxifen associated carcinoma may develop from existing polyps in symptomatic women and should therefore be removed when found. It was difficult to extrapolate current literature which mainly pertains to symptomatic women to the asymptomatic population studied and each case was therefore assessed individually to avoid exposing large numbers of asymptomatic women who have already dealt with a diagnosis of breast cancer to unnecessary anxiety and invasive procedures.

The author accepts that with current knowledge it may have been preferable to investigate pre and postmenopausal women separately from the outset as tamoxifen may affect the two groups differently.

There is no ideal screening method available as ultrasound scanning, both transabdominal and transvaginal (the main method employed in this study) gave false positive results in 46% of cases, subjecting large numbers of women to the more invasive procedures of hysteroscopy and D & C. It is also not possible from the scan appearance to predict those women who will have atrophic endometrium on further assessment from those with benign

changes. Out-patient hysteroscopy has been shown to be effective as second line investigation, being relatively east to perform and with tolerable discomfort, but is time consuming and relatively expensive. Attempts to perform pipelle biopsy, a simple out-patient procedure performed in many gynaecology out-patient clinics, was not as successful as anticipated and may have been due in part to not having ideal equipment/facilities with which to perform this. Outwith these limitations pipelle biopsy has been shown in studies to miss focal abnormalities by only sampling part of the endometrium and was shown in this study to be as uncomfortable to perform as out-patient hysteroscopy, which allows direct visualisation of the whole endometrial cavity in the vast majority of cases. It was accepted therefore not to persevere with pipelle biopsy.

It would have been ideal to have been able to sample all endometria at hysteroscopy and although this was attempted in all cases with 'apparently oedematous endometrium' this proved unsuccessful in the majority. This is an accepted limitation of the study but histology was thereafter investigated by other means.

The ultrasound appearance confirmed by this study and the difficulty obtaining endometrial specimens for histology has been previously reported. Although in the early 1990's no explanation was given for this, the histology responsible for this appearance has gained more interest in recent years, in particular from workers such as Ismail and Neven. No definitive definition exists, some considering this to be a form of atrophy and others a form of hyperplasia, but all agree the phenomenon is endometrial in origin and peculiar to tamoxifen use. This study confirmed the findings of cystically dilated endometrial glands and found low stromal cellularity with the presence or prominence of collagen within the stroma. The significant presence of collagen may account for the difficulty biopsying the endometrium and the large cystically dilated glands for the appearance of the endometrium on ultrasound scan and at hysteroscopy.

Immunohistochemistry of hysterectomy specimens from women on tamoxifen looking at ER and MIB 1 staining failed to show a significant difference in the expression of either with tamoxifen use. Although not quite significant, tamoxifen users were less likely to stain positive for stromal MIB 1 which may coincide with the low stromal cellularity found on histology, indicating a lower degree of stromal activity in tamoxifen users. Several

other modalities of action on the endometrium have been investigated including proto-oncogene expression, chromosomal changes, growth factor expression, but the cellular mechanism for tamoxifen's effect remains unknown.

Of interest, this ultrasound phenomenon would appear to be reversible in most women. This is the first study to rescan women following tamoxifen cessation and show that endometrial thickness reduces significantly off tamoxifen. A progressive effect of tamoxifen on the endometrium has been postulated by this study, whereby the initial effects of tamoxifen are reversible and can be seen as endometrial thickening on ultrasound scan without pathology, but thereafter benign abnormalities occur, polyps or hyperplasia, which may over time develop malignant foci. This clearly requires further investigation. The other interesting group of women who require further investigation is those in whom tamoxifen never affects the endometrium regardless of the time they are exposed to the drug.

The side effects experienced by women on tamoxifen rarely lead to cessation of the drug but are common and are troubesome for some women. This study reports no serious side effects but an increased incidence of hot flushes and sweats, fluid retention and vaginal discharge. Hot flushes and sweats are well documented and vaginal discharge is most likely to reflect the oestrogenic action of tamoxifen on vaginal epithelium producing a 'physiological' discharge. Women should be warned of this as, although it is common with tamoxifen use, any change in the discharge requires investigation.

Plasma levels of tamoxifen and its metabolites 4-hydroxytamoxifen, and desmethyltamoxifen were measured to assess whether metabolite levels correlated with endometrial thickness on ultrasound scan because all women receive 20mg/day of tamoxifen regardless of body mass index and this may have provided some explanation for the ultrasound findings. The initial aim had been to correlate plasma levels with endometrial abnormalities but in view of the low incidence of abnormalities detected in the study group, the aim was altered. There was a positive correlation between all metabolites and endometrial thickness but none reached statistical significance and there is little clinical significance to this finding. In addition, the relationship between tamoxifen and its metabolites and body mass index was assessed. The only outcome which reached

statistical significance was the negative correlation between 4-hydroxytamoxifen and BMI which again is of little clinical significance.

As a direct result of this study, women in Edinburgh on tamoxifen do not undergo endometrial screening but are informed of the small increased risk of endometrial cancer associated with tamoxifen use and are asked to report any abnormal vaginal discharge or bleeding which can be promptly investigated.

Tamoxifen is currently being assessed for chemoprevention in healthy women so for this group of women the results of this study are important although this study concentrated mainly on postmenopausal women. Two large trials, the Royal Marsden Trial and the BCPT-NSABP trial have been ongoing in the last few years and the IBIS trial (International Breast Intervention Study) which aimed to recruit 7000 women. The Royal Marsden Trial is studying women with a strong family history of breast cancer but has failed to show any effect of tamoxifen on breast cancer incidence in healthy women. This is unlike the BCPT-NSABP trial which showed a reduction of 45% in breast cancer frequency in healthy women such that all women are now being offered tamoxifen. The rationale for prevention trials are the lifetime risk of developing breast cancer versus the fact that tamoxifen reduces contralateral breast cancers in 30-40% of patients. The beneficial effects of tamoxifen on blood lipids, bone density and the cardiovascular system must also be weighed against the endometrial risk. There is such a large discrepancy in results from these two trials that larger numbers of women need to be involved and longer follow-up is required [Powles 1998, Lasset 1998]. The IBIS trial will be of importance to establish whether a similar reduction in the incidence of breast cancer is found [Howell 1998]. In addition the Marsden trial indicates that side effects are low and that the other oestrogenic benefits conferred by tamoxifen are present [Powles 1990, 1992].

Alternatives to tamoxifen have also been developed. More specific SERM's (Selective Oestrogen Receptor Modulators) eg raloxifene, toremifene and faslodex are currently under trial and will hopefully show fewer detrimental endometrial effects than tamoxifen. Aromatase Inhibitors, of which letrozole is one, are also promising alternatives. Letrozole has been shown to be at least as effective as tamoxifen in the treatment of breast cancer and because of its different mode of action is likely to have no long term endometrial side

effects. However, the morbidity associated with long term oestrogen suppression in pre and postmenopausal women remains to be determined.

Tamoxifen remains the drug of choice for the adjuvant treatment of breast cancer and despite its detrimental effects, the benefits from its use far outweigh its risks.

Publications Arising From This Thesis

C.D.B. Love, B.B. Muir, J.B. Scrimgeour, R.C.F. Leonard, J.M. Dixon.

Investigation of endometrial abnormalities in asymptomatic women treated with tamoxifen and an evaluation of the role of endometrial screening.

Journal of Clinical Oncology. Vol 17, No 7 (July), 1999: p2050-4.

J.M. Dixon, C.D.B. Love, L. Renshaw, C. Bellamy, D.A. Cameron, W.R. Miller,

R.C.F. Leonard.

Lessons from the use of aromatase inhibitors in the neoadjuvant setting.

Endocrine-Related Cancer. Vol 6, Issue 2, June 1999: p227-30.

C.D.B. Love, J.M. Dixon

Tamoxifen and endometrial screening (correspondence).

Journal of Clinical Oncology. Jan 14 2000: p446.

C.D.B. Love, J.M. Dixon

Thickened endometrium caused by tamoxifen returns to normal following tamoxifen cessation.

The Breast. June 2000, Vol 9: No 3.

Abstracts

C.D.B. Love, J.M. Dixon, C. Bellamy, D. Cameron, W.R. Miller, R.C.F. Leonard.

Letrozole as primary medical therapy for locally advanced breast cancer.

Breast Cancer Research and Treatment. Vol 41, No 3, 1996.

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British Journal of Surgery. Jan 1997.

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Relationship between tamoxifen duration and endometrial thickness.

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R.C.F. Leonard, J.M. Dixon.

Relationship between tamoxifen duration and endometrial thickness.

Breast Cancer Research and Treatment. Vol 46, No 1, Oct 1997.

J.M. Dixon, C.D.B. Love, J. Telford, W.R. Miller

Response to aromatase inhibitors and correlation with changes in oestrogen synthesis.

Breast Cancer Research and Treatment. Vol 50, No 3, Dec 1998.

C.D.B. Love, J.M. Dixon

Thickened endometrium caused by tamoxifen returns to normal following tamoxifen cessation.

The Breast. Vol 8, No 4, Aug 1999.

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